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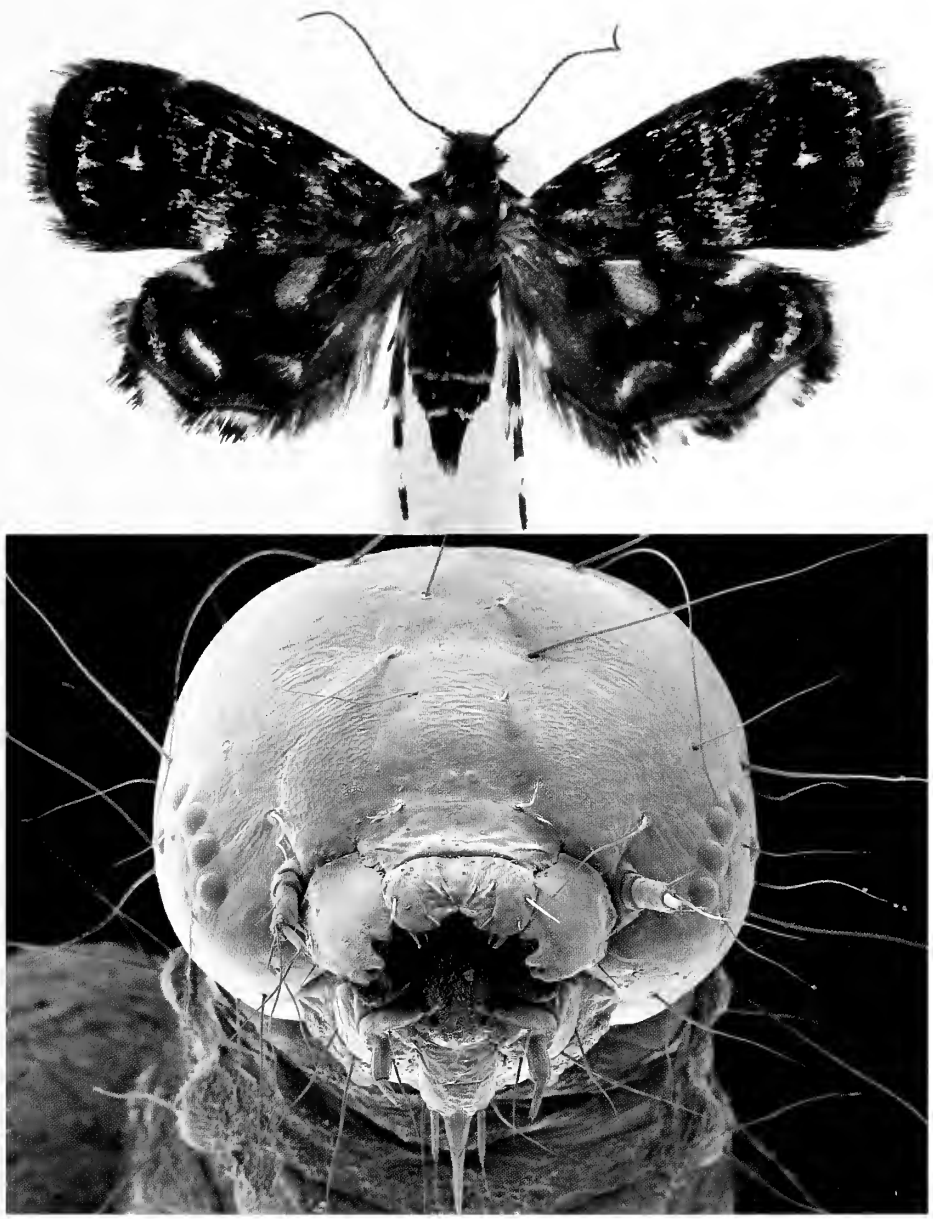

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Cover Illustration: Images of *Brethuia monolychna* Meyrick: photograph of an adult specimen (top) and an SEM of larval head (bottom). Photograph by Jadranka Rota (see article on page 121).

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IMMATURE STAGES OF METALMARK MOTHS FROM THE GENUS *BRENTHIA* CLEMENS (CHOREUTIDAE): MORPHOLOGY AND LIFE HISTORY NOTES

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ABSTRACT. In this paper the immature stages of *Brenthia monolychna* Meyrick (Choreutidae: Brenthiinae), as well as their ultrastructure, are described and figured. This is the first description of a New World brenthiine. In addition, notes on life history for four New World species of *Brenthia* Clemens are provided, including mention of their host plants and parasitoids. Host plant utilization of the genus is discussed. A clarification of the nomenclature of the longest seta on the larval abdominal segment 9 is proposed. Earlier literature disagrees on whether this is a lateral, subdorsal, or dorsal seta – my examination suggests it is the subdorsal seta 1. The recorded distribution of *Brenthia pavonacella* Clemens is questioned, and a revised distribution is suggested. Moreover, an escape mechanism, employed by all known *Brenthia* larvae, is discussed. Finally, a list of morphological and behavioral synapomorphies for the subfamily Brenthiinae and the genus *Brenthia* is provided.

Additional key words: Microlepidoptera, chaetotaxy, ultrastructure, larval escape behavior, parasitoids, Braconidae

Brenthia Clemens is a cosmopolitan genus of metalmark moths (Choreutidae). With more than 80 described species and likely an even greater number of undescribed species (Rota 2003), it is among the largest microlepidoteran genera. Together with the genus *Litobrenthia* Diakonoff, *Brenthia* is classified in the subfamily Brenthiinae (Heppner and Duckworth 1981). The relationship of brenthiines to the other two choreutid subfamilies, Choreutinae and Millieriinae, is under study (J. Rota in prep.).

Moths in the genus *Brenthia* are small – wing size ranges from 6 to 14 mm. With blue or violet metallic and white markings on dark backgrounds, wings of most species look very similar (Figs. 1–3), so much so that genitalic dissections are often necessary to confirm identification. Adults of *Brenthia* mimic jumping spiders by holding their wings to the side and above the body, and moving in rapid, jerky motion (Robinson *et al.* 1994; Rota and Wagner 2006).

Life history information is available for only a few species, mostly from Asia (e.g., Arita 1987). *Brenthia* larvae appear to be relatively specialized, one species usually feeding on a single genus or closely related genera of plants (Arita 1987; Rota 2003). The larvae of known species are surface-feeding leaf skeletonizers (Arita 1987; Aiello and Solis 2003). All known larvae seem to have a similar predator/parasitoid avoidance mechanism (see below; Williams 1951; Diakonoff 1986; Aiello and Solis 2003).

Immature stages of *Brenthia* have been described for a handful of species (e.g., Williams 1951; Arita 1987). In this paper, the larval and pupal stages of *Brenthia monolychna* Meyrick are described and figured. This is the first detailed description of the immature stages of a New World member of the subfamily. In addition, life history notes are provided on *B. monolychna*, as well as three of its congeners: *B. hexaselena* Meyrick, *B. pavonacella* Clemens, and *B. stimulans* Meyrick.

MATERIALS AND METHODS

Larvae of *B. monolychna*, *B. hexaselena*, and *B. stimulans* were collected from 2001 to 2004 in a tropical wet forest at La Selva Biological Station, a lowland reserve on the Atlantic slope of Costa Rica, Province of Heredia. *B. monolychna* pupae were reared from a collection of larvae in August 2004 also from La Selva. *B. pavonacella* larvae were collected on September 8, 2002, in Illinois, Coles County, Fox Ridge State Park, by Terry L. Harrison. Rearing was done in a laboratory in plastic bags or plastic vials filled with foliage and some soft paper tissue for control of humidity. Periods of light and dark corresponded to natural light cycles.

For preservation, larvae and pupae were placed into nearly boiling water for less than a minute and then

transferred to 75% ethanol (Zimmerman 1978). Specimens for viewing in the SEM were dehydrated in a graded ethanol series (ending in 100%), transferred to fresh solutions of hexamethyldisilazane three times, each time for approximately 15 minutes, and then immersed into fresh hexamethyldisilazane and allowed to air-dry. Dried specimens were sputter coated with gold/palladium.

A LEO/Zeiss DSM 982 Gemini Field Emission SEM was used. Photographs of adults and larvae were taken with a Nikon D100 and D1. The pupal photograph was taken with a digital camera attached to a Leica microscope, connected to a computer with Automontage® software (Synoptics Ltd., Cambridge, UK). Line art was prepared with a camera lucida. All images were edited in Adobe Photoshop CS©.

Measurements were made using an ocular micrometer. Chaetotaxy nomenclature follows Hinton (1946) and Stehr (1987). Usage of other terms is as defined in the Torre-Bueno Glossary of Entomology (Nichols 1989). Larval description is based on the last instar. Voucher specimens are deposited in the University of Connecticut Entomological Collection (UCMS).

RESULTS

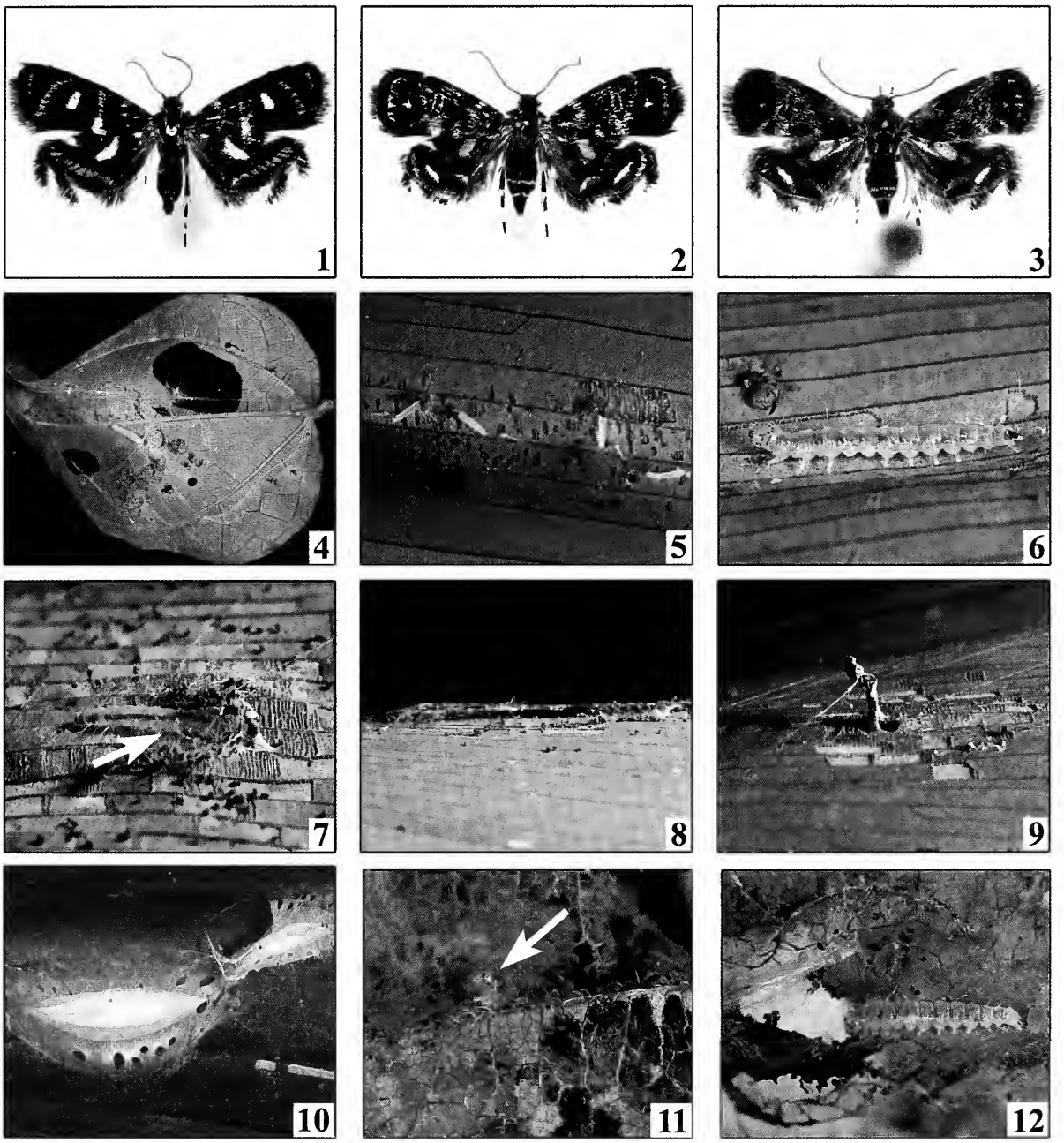
Host plants. Larvae were collected and reared on the following host plants: *B. hexaselena* larvae from *Byttneria aculeata* (Jacq.) Jacq. (Sterculiaceae) (Fig. 4); *B. monolychna* larvae from *Calathea crotalifera* S. Watson (Marantaceae), other *Calathea* spp., and from *Heliconia* sp. (Heliconiaceae) (Figs. 5–10); *B. stimulans* larvae from *Cecropia insignis* Liebm. (Cecropiaceae); and *B. pavonacella* larvae from *Desmodium glutinosum* (Muhl.) Wood (Fabaceae) and other *Desmodium* spp. (Figs. 11–12).

Morphology of the immature stages of *Brenthia monolychna* Meyrick, 1915. Larva. Pale green in life (early instars almost white) (Figs. 5–7), translucent, without any patterning on thorax and abdomen, and with long setae; average length 8.5 mm (n=5). Only primary setae present. *Head.* With dark brown spots (Fig. 6), widest at about level of P1; vertex only shallowly concave; hypognathous; setae as in Figs. 13–15; A3, AF1, and P1 extremely elongate, approaching length of head; A3 three times as long as A1, and A1 and A2 subequal; AF1 five to six times as long as AF2; S2 posteriad of stemma 1; spinneret well developed, more than twice as long as labial palpus, slender (Figs. 15, 16); stemma VI highly reduced; other stemmata arranged in a semicircle, with stemma 2 positioned slightly outside of semicircle (Fig. 14); frontoclypeus extending half way to epicranial notch; labrum notched, with toothed underside (Figs. 17, 18); mandible with well-developed teeth; hypopharyngeal spines prominent (Figs. 19, 20). *Thorax.* D1 and D2 subequal; T1 with L1 three times as long as L2 and L3, SV bisetose; T2 with L1 two times as long as L2 and three times as long as L3; T2 and T3 with SV unisetose, SD1 and SD2 subequal, L setae on separate pinacula. *Abdomen.* Setae very long, especially posteriad (Fig. 31); D1 and D2 subequal, L1 longer than L2 and L3; A1–2 and A7–9 with SV group numbering 3:3 and 2:2:1, respectively; A1 with L setae on separate pinacula; A2 with SV setae in triangle; A8 with setae D1 and D2 on common middorsal pinaculum; A9 with D1, SD1, L1, and L2 on common subdorsal pinaculum, with D1 and D2 in vertical alignment, and with extremely long SD1, corresponding in length to 4–5 abdominal segments. Prolegs long and slender, subcylindrical (Fig.21); crochets uniscrial and uniordinal; A3–6 with approximately 12 crochets arranged in mesal pennclipse (Fig. 22); A10 with 16 crochets in semicircle with posterior gap.

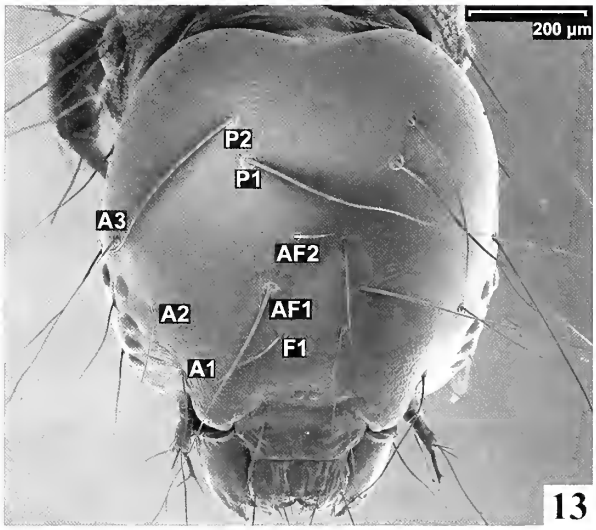
Pupa. Average length 6.25 mm (n=3); with long, fork-tipped setae (Figs. 29, 32), and short, thickly arranged, caudally oriented dorsal spines on A4–8; A4 with dorsal spines poorly developed (Fig. 23), A5 spines larger than on A4, but smaller than on A6 or A7 (Fig. 24), A6–A7 spines well developed (Figs. 25–27), A8 with field of poorly developed spines (Fig. 28). Cremaster on A10 with two pairs of slightly curved slender spines, two pairs of hook-tipped setae three times as long as spines, and two pairs of very long fork-tipped setae six to seven times as long as hooks (Figs. 29, 30).

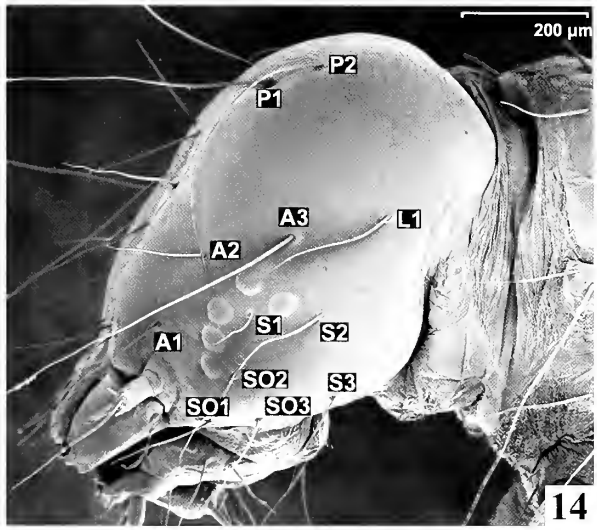
Life history notes. As in other choreutids, larvae of *Brenthia* leave diagnostic feeding damage: superficial skeletonization of undersides of leaves (Figs. 4–7). While earlier instars often feed in groups (Fig. 5), older larvae are normally solitary (Figs. 6, 7). Over the feeding area larvae create a thin, loose web into which they incorporate fecal pellets (Figs. 7, 8). Most likely, the incorporation of fecal pellets is not an active process; rather, it happens passively as the caterpillars move about the shelter and their feculae accumulate in the webbing. Larvae of all four *Brenthia* species that I have observed chew a roughly circular "escape hatch" – a wormhole – somewhere in their feeding shelter. When resting, they sit with their head next to the hole. If disturbed, larvae dash through the wormhole to the other side of the leaf (Figs. 7, 9, 11). After a little while, they wriggle through the opening backwards to their original position. Larvae of some species (e.g., *B. monolychna*) construct "fecal stalactites" (Aiello and Solis 2003) in their feeding area (Fig. 9). Located at the mouth of the escape hole on the leaf underside, these structures appear to serve as landmarks that facilitate the quick escape of a larva (see Aiello and Solis 2003). The fecal stalactites also seem to serve to suspend the webbing above the larva. *Brenthia* cocoons are often spun on the leaf undersides or somewhere on the stem and are composed of two principal parts: an inner one, which is white, fusiform, and composed of multiple layers of thick silk; and a thin, outermost silken layer that forms a sheet over the cocoon proper (Fig. 10). The whole cocoon of *B. monolychna* is less than 2 cm along its longest axis. The pupa is protruded from the cocoon at eclosion. For all four species, the development from the pupa to the adult stage took about ten days.

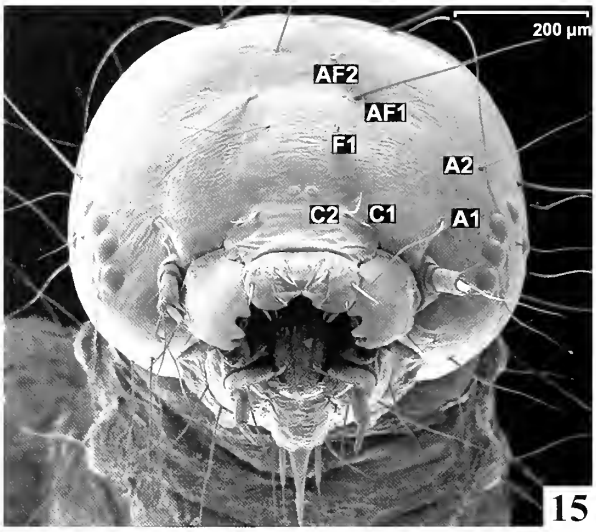
Larvae of the three *Brenthia* species reared from La Selva are heavily parasitized by braconid wasps (Hymenoptera: Braconidae) (Table 1 and Fig. 10). In *B. monolychna* the parasitism rate is close to 85% (n_{larvae}=51). In *B. hexaselena* (n_{larvae}=5) the rate is about 20%, and in *B. stimulans* (n_{larvae}=4) about 50%, but note small sample sizes. I have not reared any parasitoids from *B. pavonacella* (n_{larvae}=25).

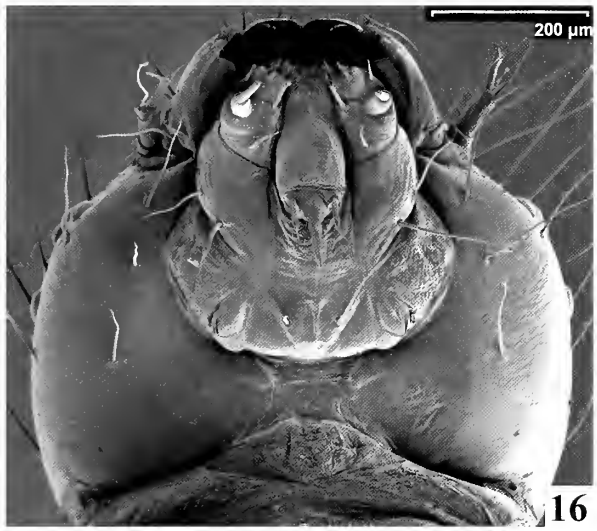


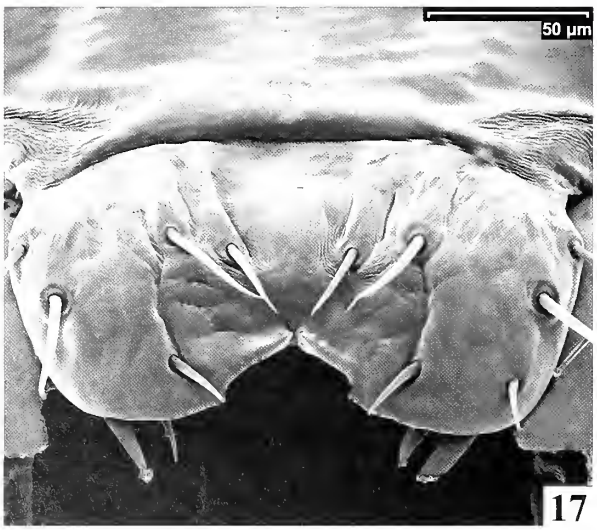
FIGS. 1–12. **1.** *B. hexaselena* adult. **2.** *B. monolychna* adult. **3.** *B. pavonacella* adult. **4.** *B. hexaselena* larva on its host plant. *B. monolychna*: **5.** Gregarious feeding of young larvae; **6.** Last instar larva; **7.** Larva going through an escape hatch; **8.** Larval webbing; **9.** Fecal stalactite; **10.** Cocoons of a healthy larva (center) and of a parasitized larva (upper right). *B. pavonacella*: **11.** Larva going through an escape hatch; **12.** Last instar larva.

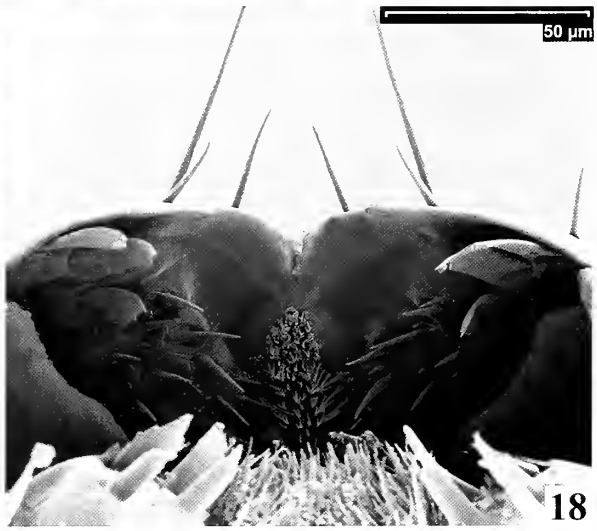




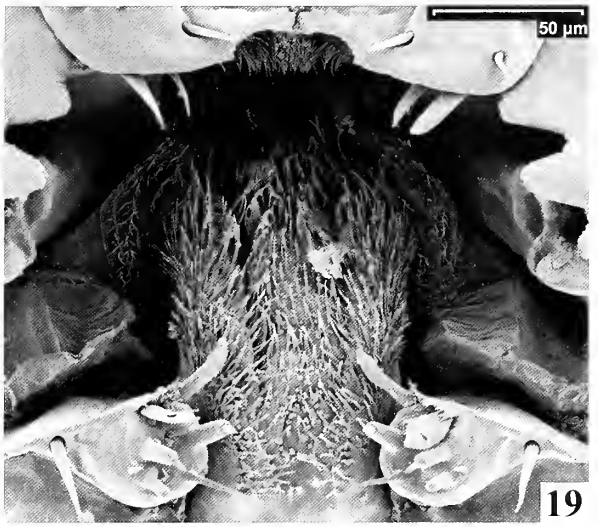


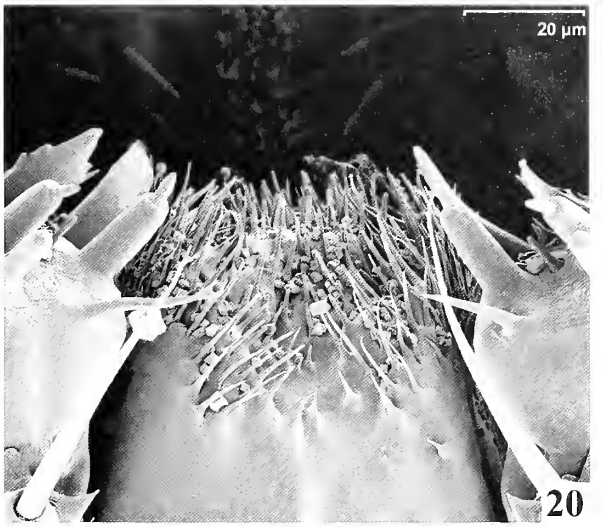


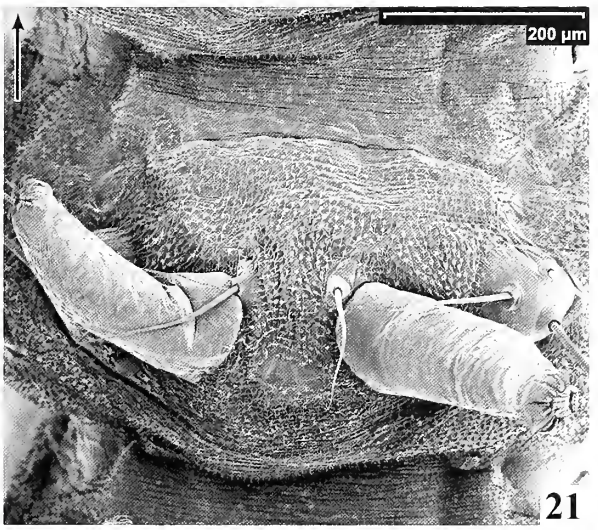


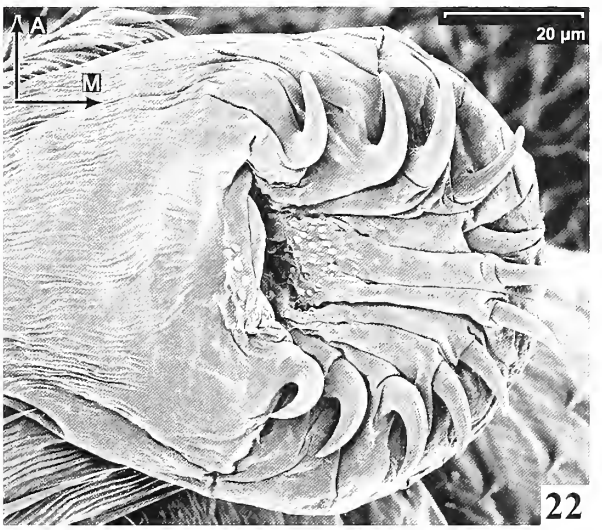


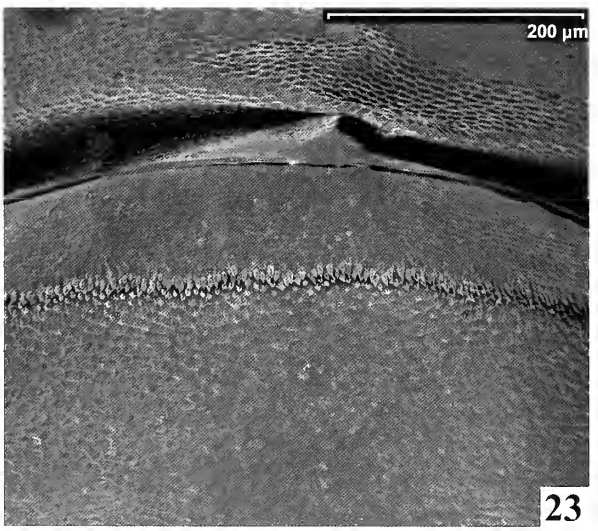
FIGS. 13–18. *B. monolychna* larva: **13.** Head, dorsal; **14.** Head, lateral; **15.** Head, frontal; **16.** Head, ventral; **17.** Labrum, dorsal; **18.** Labrum, ventral.

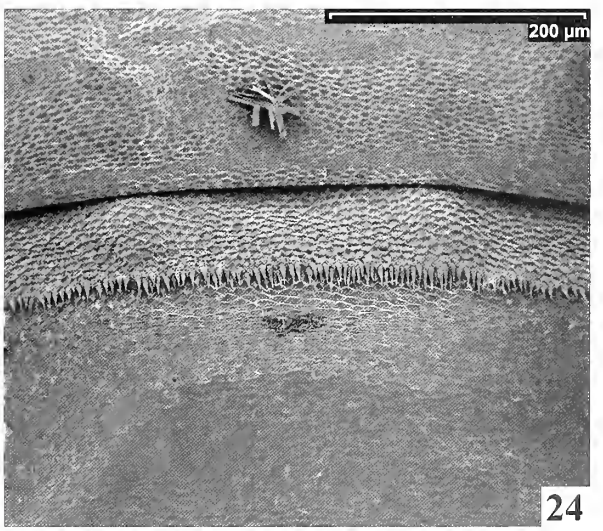




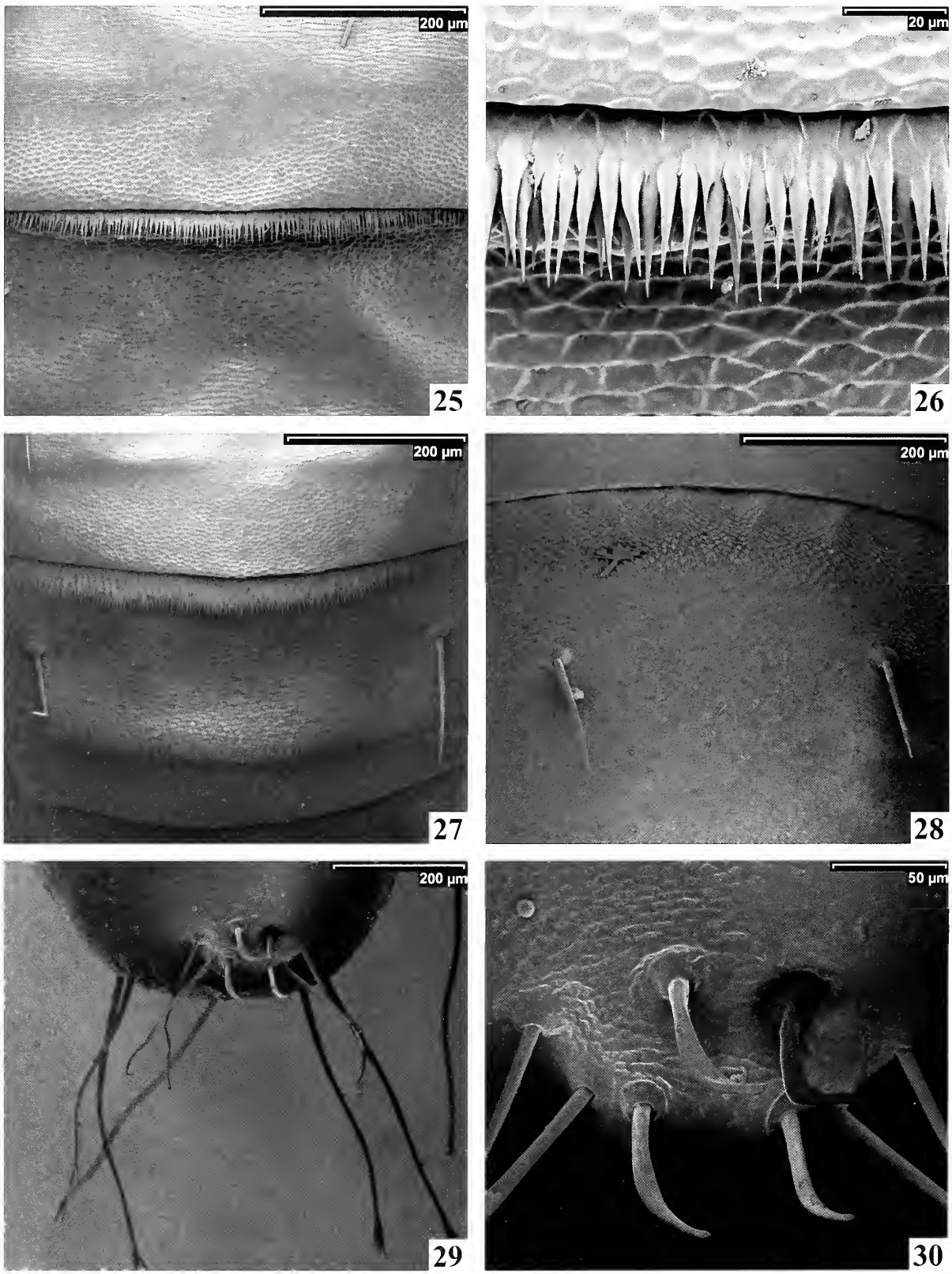




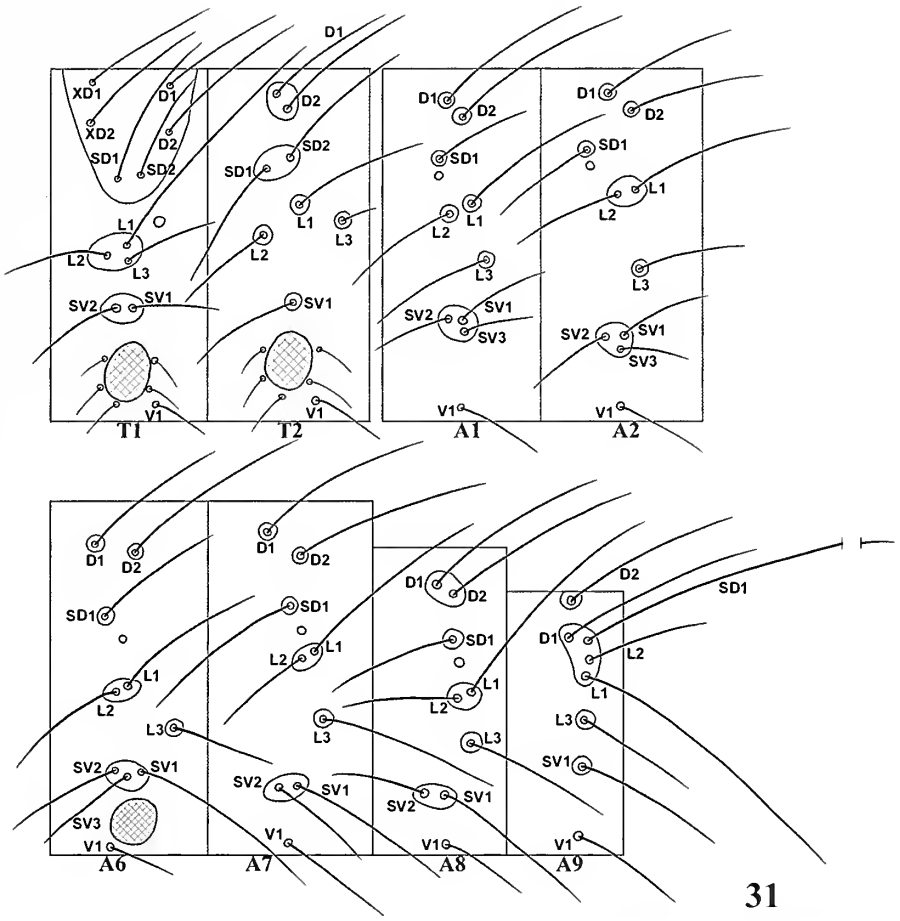


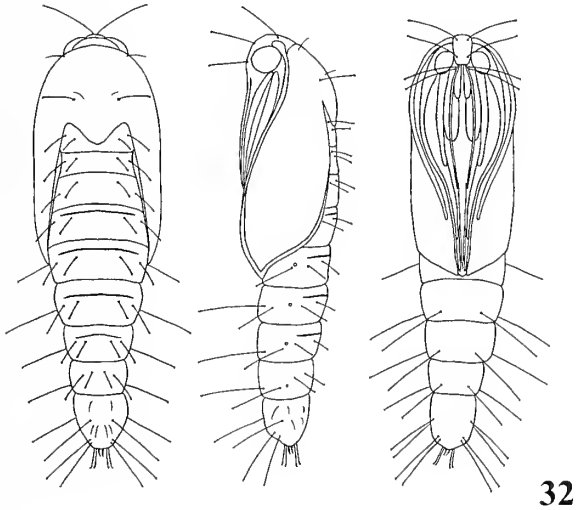


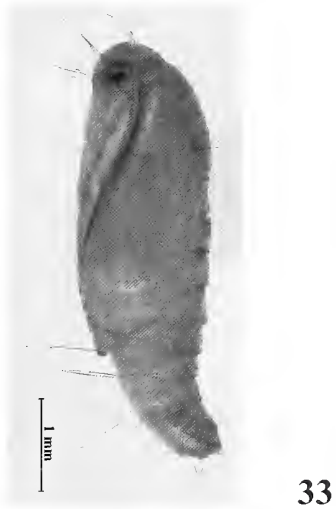
FIGS. 19–24. *B. monolychna* larva: **19 and 20.** Hypopharyngeal spines; **21.** Ventral view of abdominal prolegs (arrow points to anterior); **22.** Crochets on an abdominal proleg (arrows pointing A: anterior and M: mesad). Pupa: **23.** Dorsal spines on A4; **24.** Dorsal spines on A5.



FIGS. 25–30. *B. monolychna* pupa: **25.** Dorsal spines on A6; **26.** Close-up of dorsal spines on A6; **27.** Dorsal spines on A7; **28.** Poorly developed field of dorsal spines on A8; **29.** Dorsal view of the cremaster; **30.** Close-up of the cremaster.







FIGS. 31–33. *B. monolychna*: **31.** Larval chaetotaxy; **32.** Pupa, dorsal, lateral, and ventral view. *B. pavonacella*: **33.** pupa.

B. monolychna is an unusually abundant species at La Selva Biological Station, at least as a larva—there were traces of larval feeding damage on almost every host plant examined (n>300). The adult can be seen during the day on vegetation in the vicinity of its host plant; occasionally adults also come to mercury vapor light.

B. hexaselena is uncommon in collections from La Selva, but it can be frequently encountered during the day around stands of its host plant. I have never seen it come to lights at night (number of blacklighting nights at La Selva *ca.* 100).

B. stimulans appears to feed on mature *Ceeropia insignis* plants only – I examined dozens of saplings and never found any signs of larvae or their feeding damage. This species, most likely because its host plant is a tall tree, is rarely encountered.

Brenthia pavonaeella is the only member of the genus found in North America north of Mexico (Hodges *et al.* 1983). It is widely distributed in the eastern United States, north to southern New York (Forbes 1923). *B. pavonaeella* is often common where found. Like *B. hexaselena* and *monolychna*, it is principally diurnal.

DISCUSSION

In larvae of *Brenthia*, the longest seta on A9 is considered under different names by different authors: Arita (1987) refers to it as an additional seta of the L-group, whereas Heppner and Duckworth (1981) call it D2. Based on a careful examination of setal arrangement on all the abdominal segments and comparisons with Hinton (1946) and Stehr (1987), I agree with Williams (1951), who designated this seta as SD1.

Forbes (1923) characterized the distribution of *B. pavonaeella* as extending from New York southward to Brazil. This is almost certainly incorrect. Throughout the Neotropics there are many species of *Brenthia* with wing patterns very similar to that of *pavonacella*. My examination of genitalic characters and molecular data suggests that many superficially similar species often prove to be distantly related (Rota unpublished data). *Brenthia* collections at INBio[1] (for Costa Rica) suggest there are at least 10 species in the country, most of which are undescribed, and none of which is assignable to *pavonaeella*. Further evidence supporting this revision of the species' distribution is that *B. pavonacella* larvae are strictly limited to *Desmodium* in North America, and no Neotropical *Brenthia* have been reared from plants in this genus.

All known *Brenthia* larvae exhibit a similar escape behavior involving escaping through wormholes chewed into the floor of their feeding shelter (Aiello and Solis 2003; Williams 1951; Diakonoff 1986; this paper). While clearly this represents a predator/parasitoid escape mechanism, it is also evident that this behavior is not especially successful for avoiding parasitism by braconid wasps—as noted above, parasitism rates were as high as 85% in *B. monolychna*.

TABLE 1. Genera of braconid parasitoids from *B. monolychna* and *B. hexaselena*

B. hexaselena	Microgastrinae
	Dolichogenidea Viereck
B. monolychna	Microgastrinae
	Dolichogenidea Viereck
	Agathidinae
	Plesiocoelus van Achterberg
	Orgilinae
	Orgilus Haliday

Most species of *Brenthia* appear to be specialists—they feed on a single genus of plants or closely related groups of plants. Yet, when taking a look at the whole genus, *Brenthia* species have been recorded from a rather extraordinary array of unrelated plant families that includes both monocots and dicots: Boraginaceae (Williams 1951), Cecropiaceae (LaPierre pers. comm.; this paper), Asteraceae, Dipterocarpaceae, Euphorbiaceae (Robinson *et al.* 2007), Fabaceae (Arita 1987; Aiello and Solis 2003), Heliconiaceae (this paper), Malvaceae (Heppner 1985), Marantaceae (Aiello and Solis 2003), Moraceae (Robinson *et al.* 2007), Sapindaceae (Heppner 1985), Sterculiaceae (Hespenheide pers. comm.; this paper), Tiliaceae (Robinson *et al.* 2007), and Urticaceae (Diakonoff 1986; Arita 1987). There are also unconfirmed records of rearings from ferns (specimens in Costa Rica's INBio collection). A species-level phylogeny of the genus, at this point unattainable, would create an opportunity for the study of the evolution of *Brenthia*'s remarkable ability to exploit novel and, evidently, unrelated host plants.

A review of literature describing immature stages of 12 choreutid genera (*Brenthia* from Arita (1987), Williams (1951), and this paper; *Litobrenthia*, *Anthophila*, *Choreutis*, *Prochoreutis*, *Saptha*, and *Tebenna* from Arita (1987); pupae of *Anthophila*, *Choreutis*, *Prochoreutis*, and *Tebenna* from Patočka (1999); *Asterivora* from Dugdale (1979); *Rhobonda* and *Zodia* from Rota (2005); *Caloreas* from Keifer (1937); *Tortyra* from Wille (1937)), as well as my own investigation (unpublished data for *Choreutis*, *Hemerophila*, *Proehoreutis*, *Tebenna*, and *Tortyra*), suggest the following character states as synapomorphies

[1]INBio – Instituto Nacional de Biodiversidad

for the subfamily Brenthiinae: 1) hypognathous head (semiprognathous in Choreutinae); 2) extremely long SD1 seta on A9 (SD1 length similar to L setae in Choreutinae); 3) A9 with D1 seta present (D1 absent in Choreutinae); and 4) crochets in an incomplete circle (complete circle in Choreutinae). Likewise, the immatures of choreutine genera share numerous synapomorhies (Rota unpublished data). Together with preliminary molecular data (Rota unpublished data), these different sets of shared derived characters for Brenthiinae and Choreutinae strongly suggest that both subfamilies are monophyletic, contra Dugdale *et al.* (1998) and in agreement with Heppner and Duckworth (1981).

Character states that appear to be synapomorphies for the genus *Brenthia* are 1) crochets in a mesal pennelipse (semicircle in *Litobrenthia*), 2) two SV setae on A8 (one SV seta in *Litobrenthia* and choreutine genera except *Rhobonda*), 3) larval escape behavior through a previously made hatch, 4) two pairs of curved spines in the pupal cremaster, and 5) long forked setae on the pupa. Characters 3), 4), and 5) were not discussed in the only published description of *Litobrenthia* (Arita 1987), so at this point it is impossible to say whether these characters, or a subset of them, will prove to be synapomorphic for *Brenthia* or are in fact synapomorphies for Brenthiinae. More work is needed, especially in the Neotropics where *Brenthia* appears to be highly diverse, before we can begin answering these and other questions about this fascinating group.

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OVIPOSITION STRATEGY AND BEHAVIOR OF THE KARNER BLUE BUTTERFLY, *LYCAEIDES MELISSA SAMUELIS* (LYCAENIDAE)

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ABSTRACT. Lepidoptera are known to oviposit on host-plants singly or gregariously, and eaeh particular strategy is thought to aide in larvae survival. We studied the oviposition strategy of the Karner blue butterfly (Lyeaenidae), *Lycaeides melissa samuelis* Nabokov, which has two distinct broods per year. We found the Karner blue butterfly oviposition strategy differed between the two broods, but feeundity did not differ. The first brood primarily oviposited singly on host-plants (1.06 eggs/loeation), whereas the seeond brood oviposited in clumps (2.94 eggs/loeation). From these data and noted behavior observations, we hypothesize that the ehange of strategy is due to environmental eonditions and the risk associated with larval aggregation.

Additional key words: host-plant quality, gregarious, larvae, fecundity, risk

The ecology of oviposition behavior in Lepidoptera has been intensively studied from an ecological and evolutionary perspective. Host-plant quality is well known to affect butterfly oviposition preferences and behavior (Grossmueller & Lederhouse 1985; Myers 1985; Singer 2003). Additionally, several studies have focused on the evolutionary causes, benefits, and risks of having gregarious eggs and larvae (Stamp 1980; Courtney 1984; Inouye & Johnson 2005). However, we are not aware of any study which has examined a species that changes oviposition behavior with their particular generation. Data on such behavior changes may provide insights into the trade-offs of ovipositing singly or in larger clumps. Behavior changes may also reveal environmental conditions that correlate with particular oviposition strategies.

The federally endangered Karner blue butterfly (Karner blue) (Lycaenidae), *Lycaeides melissa samuelis* Nabokov, is an inhabitant of oak savanna and pine barrens in the Midwestern and Eastern United States. The Karner blue feeds exclusively on wild blue lupine, *Lupinus perennis* L. (Fabaceae), always has two broods per year, and has a mean adult lifespan of 3.5 days (Knutson *et al.* 1999). The life cycle of the Karner blue is described in Figure 1. Throughout the Karner blue range, dry conditions are relatively common during the second brood larval phase, and host-plant senescence at this time decreases leaf nitrogen and water in host-plants (Pickens & Root 2008). The result is likely to be increased second brood larvae mortality and/or poor physical condition of adults. In this study, we report the Karner blue oviposition strategy, oviposition behavior, and estimate the fecundity of females for each of the two broods.

MATERIALS AND METHODS

Our study took place at four sites in Lucas County, Ohio, USA and the sites were located at The Nature Conservancy's Kitty Todd Preserve (41° 37' N, 083°47' W). The four sites were separated by distances ranging from 75 to 660 meters. The plant community was a black oak/lupine savanna as described by NatureServe (2006). Dominant woody vegetation included *Quercus velutina*, *Quercus ellipsoidalis*, and *Quercus alba* with a tallgrass prairie herbaceous layer.

We used modified Pollard-Yates transects (Thomas 1983; Pollard & Yates 1993) to survey Karner blues daily for the first and second brood in 2005. One to three trained observers performed Karner blue surveys throughout all host-plant areas at the four sites. When a female Karner blue was observed, we performed a 15-minute behavior observation. During the second brood, 16 of 121 observations were performed for only 10 minutes since a larger population of butterflies was anticipated. If a butterfly was demonstrating obvious oviposition behavior after 15 minutes, we continued the observation for three additional minutes. We recorded oviposition locations, total time of observation, number

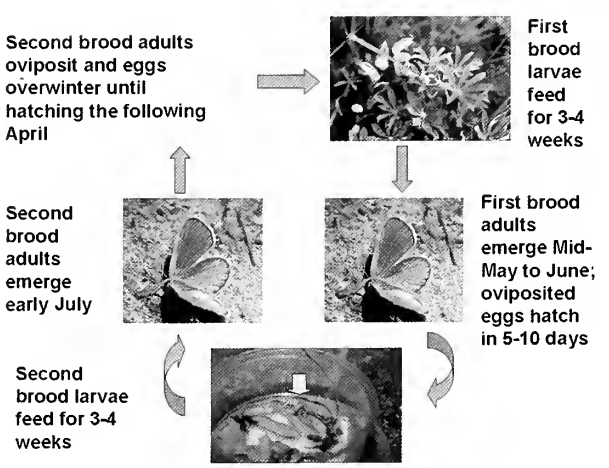

FIG. 1. Life eycle of the Karner blue butterfly, *Lycaeides melissa samuelis*. Photograph of adult male by Mareus Rieei.

of ovipositions, and substrate of oviposition. Since individual *L. perennis* are difficult to distinguish in the field (Grigore & Tramer 1996), a single location was recorded if eggs were within the same 1 m^2 area. We acknowledge that our definition of clumped differs somewhat from studies where larvae are certain to directly interact with each other. However, plants within 1 m^2 experienced similar environmental conditions (e.g. shade, moisture) and might have even been the same individual plant.

Two HOBO temperature data loggers (Onset Computer Corporation, Bourne, MA) were placed in an open, sunny area and a well-shaded area at one study site. Temperatures were recorded every 40-minutes and were correlated with survey and oviposition dates and times. SAS 8.01 (SAS Institute 2000) was used for data analysis, and means are reported with ± standard error.

RESULTS

Oviposition Behavior Description. During hot and dry weather periods late in the Karner blue's first brood, females were commonly observed alighting on host-plant leaves and moving in a tight circle while batting their antennae against *L. perennis* leaves. The butterflies were then observed either to crawl down the stem and oviposit or move to another host-plant. For the latter, oviposition often occurred after alighting on 2–5 different *L. perennis*. This circling behavior was much more prevalent late in the first brood compared to other periods, although we did not quantify the exact frequency of the behavior. The circling behavior has previously been observed in a captive setting and is suggested to be a method used by the butterfly to cool itself (Lane 1999). In contrast to Lane's observations, the pattern we observed was inconsistent with heat being the sole factor causing the behavior. The second brood adult period in July was much hotter than the first brood (unpublished temperature logger data), and yet the circling behavior was rarely observed with second brood adults. In contrast, second brood adults went straight to host-plants and selected locations to oviposit without circling on individual leaves. During the first brood of Karner blues, ovipositions were only on lupine stems. For the second brood, we observed Karner blues ovipositing on lupine (*L. perennis*) (79.8%), grasses (16.7%), dewberry (*Rubus villosus*) (1.2%), early goldenrod (*Solidago juncea*) (1.2%), and on the ground (1.2%).

Oviposition Strategy and Rate. We observed 46 ovipositions in 58 observations for the first brood and 84 ovipositions in 122 observations for the second brood. The frequency of oviposition per observation (1 or 0) differed between Karner blue broods. The first brood oviposited at least one egg per observation more often than the second brood (Fisher's exact test, df=1, χ^2=10.6, p<0.002, 45% vs. 21%). However, when Karner blues did oviposit, the mean number of ovipositions per location (within 1m^2) for the second brood was greater than the first brood (Wilcoxon 2-sample test, n=51, 2.94 ± 0.30 vs. 1.06 ± 0.04 eggs/location, Z=-5.24, p<0.0001). Essentially, eggs were oviposited individually in the first brood and oviposited in clumps more often during the second brood.

We used Lane's (1999) female Karner blue movement threshold of 24.6°C and assumed Karner blues oviposited at temperatures above this threshold. From the temperature loggers, we estimated the number of hours available for oviposition behavior was 10.7 hours/day for the first brood and 11.3 hours/day for the second brood. Multiplying by the oviposition rate for each brood, we estimated each female oviposited 34.9 eggs/day for the first brood and 34 eggs/day for the second brood. Since Karner blues have a mean adult lifespan of 3.5 days (Knutson *et al.* 1999), we estimated 139.6 eggs/female for the first brood and 136 eggs/female for the second brood.

DISCUSSION

Our study found that Karner blues changed oviposition strategy and behavior based on their generation, which may also correlate with environmental characteristics. Lepidoptera species are known to oviposit using either a clumped strategy or by ovipositing singly, but generally not both strategies. The first brood of Karner blue adults distributed their ovipositions as much as possible by primarily ovipositing eggs singly on host-plants, while the second brood adults oviposited 1–6 eggs per 1 m^2. Nevertheless, a similar number of eggs were produced for both broods. These results complement previous research, which show Karner blue population growth rates are more density-dependent for the first brood adults (i.e. higher first brood abundances lead to lower per capita successful reproduction) compared to the second brood adults (Pickens 2007). If lupine is a limiting factor, the host-plant should be more limited for the first brood adults because it appears optimal for Karner blues to spread their ovipositions on many lupine plants during the first adult generation.

There are two distinct possibilities for the observed difference in oviposition strategy between broods. First, Karner blues are known to be vulnerable to droughts in June-July (Maxwell 1998; Pickens 2007), so the eggs could be spread as much as possible by the first brood adults to avoid a catastrophe caused by the senescence

of a small group of host-plants. The trade-off involves the disadvantage of expending more time and energy to oviposit and the advantage of optimal oviposition placement. Time for oviposition is crucial because researchers have found butterflies to be more limited by time for oviposition rather than the number of eggs in the oviduct (Courtney 1984; Doak *et al.* 2006). Therefore, we would expect the single oviposition strategy to be advantageous for second brood larvae survivorship. The circling behavior observed could be attributed to butterflies searching for plants with a higher water content and later senescence. Of course, host-plant nitrogen or water content is unlikely to be selected for by second brood adults since their eggs do not hatch until after the winter season.

A second hypothesis for a differing oviposition strategy is that the survivorship of eggs through the nine month overwintering stage could be less than the survival of eggs for the 5–10 day period in summer. For example, three or four feeding larvae could compete for host-plant foliage on the same host-plant, but if only 1 of 4 eggs survive through the winter, there would be no larval competition. Each of the two hypotheses is plausible and a combination of these two theories could also have lead to the observed behavior of the species. Future studies could assist in determining if this behavioral strategy is a response to host-plant conditions, which differs between the two broods.

Our oviposition rate estimates are limited by a one year study period. However, fecundity for Karner blues has only been reported for individuals taken from the wild without knowledge of age or nutritional conditions. Our methodology found fecundity of females in the field (139.6 or 136 eggs per female) to be comparable to the number of eggs produced by females brought into captivity, which has been noted at a maximum of 200 eggs, but is usually closer to 100 eggs (unpublished reports, Toledo Zoo). In conclusion, we have documented oviposition rates of a bivoltine species and we found a shift in Karner blue oviposition strategy between the two broods which may indicate a relative survival advantage for the species.

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LIMITS ON THE WAVELENGTHS REPRESENTED IN ULTRAVIOLET IMAGES OF LEPIDOPTERA

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ABSTRACT. Ultraviolet (UV) imaging is commonly used in the study of plant and animal coloration, especially for visualizing the size and shape of UV reflectance patterns in butterflies and moths. We show that the spectral emission of light sources and the transmission spectra of the lenses and filters often used to make such images are not flat in the UV. As result the images are made with a narrow range of UV wavelengths as small as 360-390 nm in an inexpensive system with typical components. This limit on the wavelengths represented in the images can lead to various measurement errors that must be considered in using such images to characterize and study UV coloration.

Additional key words: coloration, photography, image analysis

Photographic images that are produced using only near ultraviolet (UV) wavelengths of light (300–400 nm) have often been used to visualize the size, shape, and brightness of UV components in the coloration of butterflies (recent e.g.'s: Obara and Majerus 2000; Knüttel and Fiedler 2001; Robertson and Montiero 2005; Rutowski *et al.* 2007; Fig. 1), moths (recent e.g., Lyytinen *et al.* 2004), and other organisms (recent e.g.'s: crustaceans, Ziel and Hofmann 2001; fish, Cummings *et al.* 2002; lizards, Thorpe and Richard 2000; birds, Bleiweiss 1994; plants: Yoshioka *et al.* 2005). Ideally such images are made with systems that are optimized for UV imaging with quartz lenses and filters, and broad-spectrum UV light sources (Eisner *et al.* 1969). However, such equipment is expensive and, in the case of quartz camera lenses, becoming rare, which has led those interested to seek less expensive options (Ferris 1972; Hill 1977). Indeed, relatively inexpensive digital still and video cameras with glass lenses have displayed a sensitivity to UV wavelengths that is adequate for producing such images with both sunlight and light from other sources (e.g. Acorn 2002; Rutowski *et al.* 2007).

Unfortunately, the specific wavelengths that contribute to these images have not been quantified. Given the variation in spectral output of different light sources and in the transmission properties of the lenses, filters, and fiber optics often used in such systems, there are good reasons to think that even with quartz optics, some UV wavelengths are better represented than others in these images, potentially confounding their interpretation. For example, and as Hill (1977) pointed out, glass does not transmit light at wavelengths less than 350 nm, which yields images that are based on a very biased sampling of UV wavelengths. This bias could impede accurate characterization of color pattern features in the UV that many invertebrate and vertebrate animals would be able to see (Briscoe and Chittka 2001; Bennett and Cuthill 1994).

Here we present information regarding the spectral properties of the output of several light sources we and others have used, and the transmission properties of lenses and filters employed in a non-quartz imaging system. Our goal was to assess quantitatively the spectral composition of light reaching the light-sensitive elements in such systems, and therefore the potential biases imposed by this equipment on UV images.

METHODS AND RESULTS

Characteristics of light sources. We evaluated three light sources for the intensity and spectral composition of the light they emit. The relative intensity of near UV light emission (300–400 nm) was measured using Ocean Optics OOIIRAD (v. 2 beta) software. We first calibrated our spectrometer (Ocean Optics USB-2000) for irradiance measurements with a bare 400 ϕm diameter optical fiber using a calibrated light source (Ocean Optics LS-1-CAL). For these measurements, we attached a collimating lens (Ocean Optics UV-74 adjusted to an acceptance angle of approximately 3.5°; flat transmission spectrum) to the optical fiber that had been used to calibrate the spectrometer, and positioned this fiber/lens so that it was 5 cm from and pointed directly at the surface of each bulb. The software was set to spectral graph mode ($\phi W/cm^2/nm$) and an emission spectrum was captured.

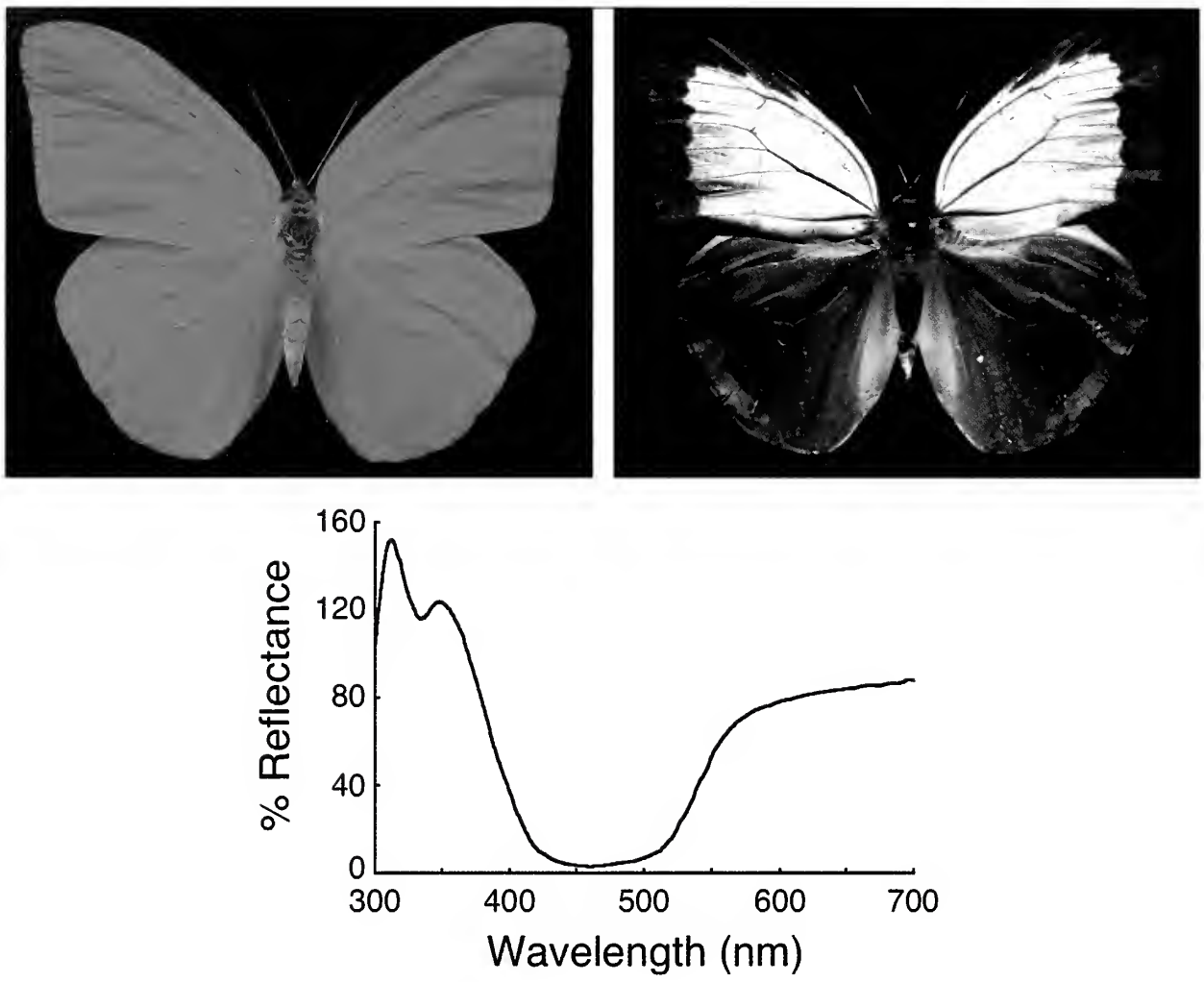

FIG. 1. Images of a male Phoebis agarithe made (above, left image) with wavelengths of light visible to humans (400–700 nm) and (above, right image) with near UV wavelengths using the system described in Fig. 4 with the straight tube fluorescent light source. The graph below shows the reflectance spectrum relative to a white standard taken from the central UV-reflectant area of the forewing.

Dark noise readings were taken prior to each measurement and subtracted from the bulb emission recordings.

Fluorescent UV light, ring tube (Sylvania 350 Blacklight 22W). Peak output of this bulb was around 340 nm but fell to 0 at 300 nm and below (Fig. 2). Two narrow, low amplitude spikes occurred in the spectrum between 400 and 450 nm. Among the light sources we examined, this source produced the highest level of UV emissions for the area of bulb sampled.

Fluorescent UV light, straight tube (length: 30 cm; Lunalite® Blacklight, IMS Corporation). The output of this tube was very similar to that of the ring tube in the UV but with only a few small peaks greater than 400 nm (Fig. 2).

Tungsten filament light with fiber optic guides (Fiber-lite Model 180 with twin gooseneck Fiber-light guides and 21V 50W EKE bulb, Dolan Jenner Industries, Inc). The output of this light source peaked around 650 nm and declined rapidly on the long wavelength side of the peak (Fig. 2). There was very little UV light in the output with essentially none below 360 nm.

Light path transmission properties. In the set up we have often used to create UV images (e.g., Rutowski *et al.* 2007), the path from the light source to the specimen and then to the photodiode in the camera includes two filters and a lens (Fig. 3). One filter ("UV pass") is mounted at the front of the camera lens and should pass only wavelengths below 400 nm (UV) and above 700 nm (infrared (IR)). The other filter is placed between the light source and the specimen and should block IR wavelengths. Our experience as well as that of

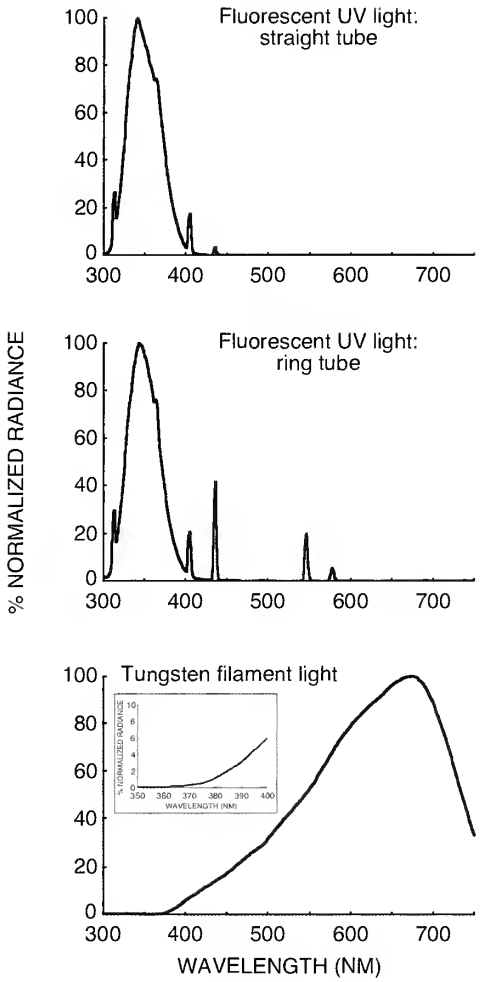

FIG. 2. Spectral properties of the output of the three light sources used in UV imaging. The inset for the tungsten filament light source shows its output in the UV with the intensity scale expanded. See text for details.

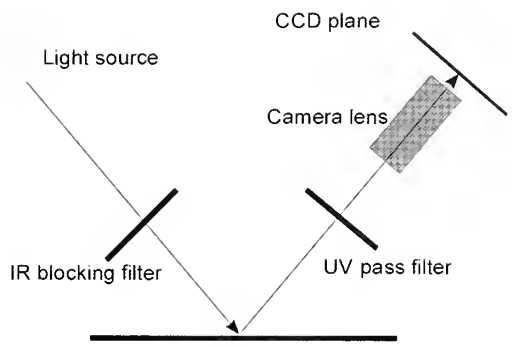

FIG. 3. Light path diagram for a typical UV imaging system.

others (http://dpfwiw.com/filters.htm#ir; last accessed 21 January 2008) suggests that the IR wavelengths passed by the UV pass filter, especially from IR rich light sources such as tungsten filament lamps and the sun, interfere with making images using only UV light. Hence, at least with the tungsten filament source, we place a filter in the light path to remove infrared energy. Others place it on the camera lens with the UV-pass filter.

With spectrophotometric equipment we evaluated the transmission properties of each of these elements in turn (Fig. 4). A light beam from a xenon lamp (Ocean Optics PX-2) was passed through the optical fiber/collimating lens arrangement described above, and was oriented normal to and 5 mm above the element whose transmission was to be measured. The element was held with a clamp 10 cm above a white standard (a slide coated with magnesium oxide), such that the beam from the PX-2 passed through and was focused onto the standard. An identical optical fiber/collimating lens setup was positioned 45° relative to and focused on a spot within the circumference of the PX-2 beam striking the white standard. This collected light was passed into the spectrometer and measured using Ocean Optics OOIBASE32 software. The element (lens or filter) then was removed from the light path and a second reading was taken from the beam striking the standard. Dark noise was removed prior to taking each measurement. We calculated the transmission characteristics of the element by taking the difference between these two reflectance spectra.

UV pass filter (Tiffen Series 7 18A). The transmission spectrum displayed a clear peak around 360 nm but dropped to essentially zero at 300 and 400 nm (Fig. 4). No measurable light was transmitted by this filter between 400 and about 710 nm. However, some infrared wavelengths were passed as is evident from

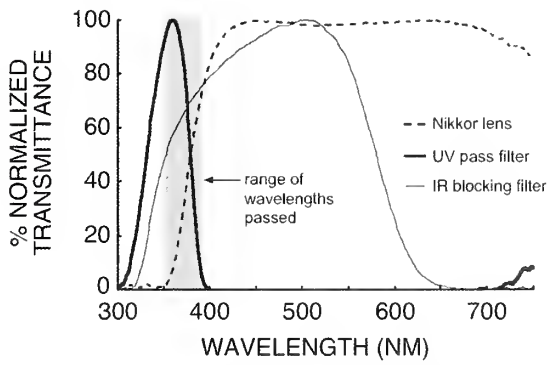

FIG. 4. Transmission spectra for elements in the light path used for UV imaging. Shaded range of wavelengths is that that would be passed through a system with these elements. See text for details.

10% transmission for this filter at 740–750 nm.

IR cut filter (Edmund Scientific). This filter displayed a single broad transmission peak with maximum transmission around 500 nm (Fig. 4). However, essentially no light energy was transmitted below 320 nm or above 650 nm.

Camera lenses. We have used two lenses for UV imaging. One lens is an AF MicroNikkor f-2.8 (Nikon), whose transmission rose quickly above 350 nm to reach approximately 95% at 420 nm, and remained largely flat at nearly 100% transmission to about 685 nm before declining slowly to approx. 85% at 750 nm. The other lens we examined was a Takumar f-1.8, 55 mm (Pentax screw mount, Asahi Optical Co.), whose transmission spectrum (not figured) was similar to that of the Nikkor lens, except that transmission rose more slowly above 350 nm, did not reach 100% transmission until about 525 nm, and began to decline slowly from 550 nm to approx. 85% at 750 nm.

From the graph summarizing the transmission characteristics of all these elements, we can see how the intensity of transmitted of light might vary with wavelength in the light path containing these elements (Fig. 4). Little or no energy at wavelengths below 350 or above 400 were passed by this combination of filters and lens. In fact, the peak of light transmission occurred between about 370 and 380 but transmission dropped rapidly to zero on either side (Fig. 4, shaded area).

DISCUSSION

Our analysis of the emission of several commonly-used light sources and the transmission properties of filters and lenses that might be used in UV photography suggests that images produced with relatively inexpensive systems are made with a very narrow range of wavelengths, namely 360–390 nm. Internal features of the camera used may also set limits on the range of wavelengths that will contribute to the image. In digital cameras, the light-sensitive diode array has its own spectral sensitivity function (Stevens *et al*. 2007). However, because this sensitivity is generally broad and extends into the UV and IR, many manufacturers cover the diode array with filters that block these wavelengths, especially those in the IR, to reduce chromatic aberration and make the picture clearer. We did not take these filters into consideration in our analysis and for the most part they are not thought to have much impact on the UV wavelengths (http://www.astrosurf.com/buil/d70/ircut.htm; last accessed 8 January 2008). In film cameras, the various films available vary significantly in their sensitivity to UV wavelengths (Ferris 1972; Hill 1977).

What sorts of problems could arise from failing to take into consideration this variation in light source UV emissions and the UV filtering properties of the light path elements? Errors would arise if the wavelength of peak UV reflection of the specimen is some distance from the wavelength of peak transmission of the imaging system used. We propose three problems that might result from light source and equipment transmission biases in recording UV reflectance of biological materials: 1) failure to detect a bright UV signal that is present, 2) underestimation of relative signal brightness, and 3) misrepresentation of the area and shape of UV pattern elements.

As a case in point, we imaged the iridescent UV reflectance from the dorsal wing surface of a male sulphur butterfly, *Eurema candida*, which led us initially to conclude that the UV reflectance was quite weak. However, subsequent spectrophotometric studies showed clearly that the UV signal was bright with a high peak (>60%) at about 340 nm, but exhibited only about 20% reflectance at 370–380 nm (Rutowski *et al*. 2007), namely, those wavelengths used to make the images. We note that even if a grayscale reference were included in the image (e.g. Knüttel and Fiedler 2000, 2001), it would be subjected to the same filtering as the butterfly image and so would still underestimate the brightness of the male's coloration. The highly unequal transmission of light across the UV wavelengths by imaging systems also needs to be carefully considered when using images for color analyses, such as those recently outlined by Stevens *et al*. (2007).

Quartz optics are transparent to UV wavelengths with a flat transmission spectrum between 300 and 400 nm. This will help broaden the range of wavelengths available in the UV to make images. However, the UV-pass filters that are required to block longer wavelengths such as the Tiffen filter we used, the Hoya UV360 (e.g. Obara and Majerus 2000; for transmission spectrum, see: http://www.hoyaoptics.com/pdf/U360.pdf; last accessed 8 January 2008), and the Schott UG1 (Knüttel and Fiedler 2000; for transmission spectrum, see: http://www.schott.com/optics_devices/filter/english/index.html; last accessed 8 January 2008) do not have a flat transmission spectrum in the UV but show a peak in transmission with steep sides in the middle of the 300–400 nm range. This limits the benefits of using quartz optics.

Also, light sources such as those used here do not emit equal intensities of UV at all wavelengths. Even sunlight contains a decreasing amount of ultraviolet energy as wavelength decreases from 400 to 300 nm. Moreover, the spectral quality of sunlight varies with moment-to-moment atmospheric changes in cloud

cover and to a lesser degree changes in sun position (List 1951).

In summary, UV imaging is certain to remain one of the primary techniques used for assessing the shape and size of UV color pattern elements. Even with the limitations discussed here UV imaging is a powerful and quick qualitative technique for assessing characteristics of UV color patterns in animals and plants. However, for those using it to quantify color signals, we make three recommendations. First, the spectral properties of the light sources, filters, and lenses used should be carefully taken into consideration in interpreting any aspect of resulting images. Second, as much as is practically and economically possible, we recommend the use of equipment that maximizes the range of UV wavelengths that are contributing to the resulting images. Finally, any effort to quantify the reflectance properties of UV signals of interest should couple the use of images with full-range spectrophotometry.

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NECTAR FLOWER USE AND ELECTIVITY BY BUTTERFLIES IN SUB-ALPINE MEADOWS

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ABSTRACT. Nectar flowers are an important resource for most adult butterflies. Nectar flower electivity was evaluated for the pierid butterflies *Pontia occidentalis* (Reak.), *Colias nastes* Bdv., *Colias christina* Edw., *Colias meadii* Edw., *Colias philodice* Godt., and *Pieris rapae* (L.), and the nymphalid *Nymphalis milberti* (Godt.). Butterflies were observed in a series of sub-alpine meadows in Kananaskis Country, Alberta, Canada. A total of 214 observations of nectar feeding were made over four years. The butterflies were found to nectar on a range of species of flowering plants. Despite the variety of flower species used, there was relative consistency in use among butterfly species. Tufted fleabane (*Erigeron caespitosus* Nutt.) and false dandelion (*Agoseris glauca* (Pursh) Raf.) were the flowers most frequently elected by these butterflies.

Additional key words: Alpine, habitat quality, preference, Pieridae, Nymphalidae, host plants, Kananaskis Country, Alberta

INTRODUCTION

For most species of butterflies, nectar is the main source of food energy in the adult stage. Access to nectar resources can affect many aspects of the ecology of butterflies. For example, flowers have been shown to affect the movement of butterflies. Butterflies often disperse to areas or patches with an abundance of nectar flowers (Peterson 1997; Brommer & Fred 1999; Matter & Roland 2002). Similarly, butterflies may emigrate from areas low in nectar resources (Kuussaari *et al.* 1996), although emigration and immigration need not respond in kind, even to the same resource (Matter & Roland 2002). These changes in movement patterns can in turn affect local abundance and potentially population growth.

Nectar resources may also directly influence population growth. For species that continue oogenesis during the adult stage (Boggs 1997), lifetime fecundity can increase with the amount and quality of nectar (Murphy *et al.* 1983; Fischer & Fiedler 2001; Mevi-Schütz & Erhardt 2005). For species that do not continue to mature eggs as adults, nectar may have a positive effect on fecundity by increasing lifespan and decreasing egg resorption (Boggs & Ross 1993).

Despite the importance of nectar for butterfly ecology, nectar flower use by individual species is often poorly known, particularly in specific localities. Nectar flower use can vary by region and can depend on the availability of flowers and on relative nectar quality (Scott 1986). Nectar species use and, in particular electivity, is an important aspect of habitat quality. Use of a flower species only indicates that a butterfly may acquire resources from that species. On the other hand, electivity indicates that a species chooses or "elects" to feed on particular species in greater frequency than its availability. Thus, electivity may indicate that a nectar resource is particularly valuable having appropriate viscosity, sugar content, amino acids or other nutrients. Alternatively, an elected resource may simply be enticing without offering any substantial or consistent benefit. Here, we examine nectar flower use and electivity by several species of butterflies within sub-alpine meadows in the Rocky Mountains of Alberta, Canada.

MATERIALS AND METHODS

Study site. Nectar feeding observations and flower surveys were conducted during the summers of 2003 to 2006 in 17 meadows along Jumpingpound Ridge, Alberta, Canada (51°57'N, 114°54'W). The meadows are at tree-line (~2500 m) and are comprised of grasses, sedges, mountain avens, and many other species of wildflowers. The lower slopes of the meadows are bordered by forest consisting of *Pinus contorta* Dougl., *Abies lasiocarpa* (Hook.) Nutt., and *Picea engelmannii* Parry ex Engelm., which may be a barrier to the dispersal of some species (Ross *et al.* 2004).

Study species. The butterflies examined in this study all inhabit sub-alpine meadows and use the flowers occurring there as nectar sources. Each species depends on the meadows to a varying degree. For some species, both host plants and nectar flowers are only present within the meadows. Other species have host plants and nectar flowers occurring in the meadows and elsewhere. Some species only use these meadows for nectar flowers and hilltopping as their larval host plants occur in other habitats.

Nymphalis milberti – Eggs are normally laid on nettles (*Urtica* sp.) Nettles are not found in the meadows we studied. There are dubious reports of larvae on *Helianthus*, *Ulmus*, and *Salix* (Bird *et al.* 1995). A variety of flowers as well as rotting fruit and tree sap have been reported as adult energy sources in

other areas (Austin & Austin 1980; Iftner *et al.* 1992; Reed 1997). This species uses the meadows primarily for nectaring; *Salix glauca* L. is found in the meadows and is a possible, but unlikely, host plant.

Colias christina – Fabaceae in the genera *Hedysarum*, *Lupinus*, and *Thermopsis* are reported host plants for this butterfly (Bird *et al.* 1995; Guppy & Shepard 2001). *Hedysarum sulphurescens* Rydb. is common in these meadows and is a likely host plant. To our knowledge, nectar flowers for this species have not been reported.

Colias philodice – Larvae use a variety of herbaceous Fabaceae, particularly *Trifolium* spp. and *Medicago sativa* L. as host plants (Scott 1986; Bird *et al.* 1995; Guppy & Shepard 2001). A range of flowers, mainly legumes and asters, has been reported as nectar sources (Shields 1972; Iftner *et al.* 1992). This species uses the meadows for nectaring and legumes occurring in the meadows likely are used as host plants.

Colias meadii elis – Larvae of *Colias meadii elis* feed on Fabaceae found in low alpine meadows and valleys in the Rocky Mountains of Alberta and British Columbia. Roland (1982) reports *Erigeron aureus* Greene and *Tonestus* (=*Haplopappus*) *lyallii* (A. Gray) A. Nelson as preferred nectar flowers at a near-by location. In Colorado, Watt *et al.* (1974) detail nectaring of *C. meadii* on several species in the Asteraceae. This species is a meadow resident using local plants for both larval and adult resources. At our site, oviposition on *Astragalus miser* has been recorded (B. Christian Schmidt, personal observation).

Colias nastes – *Astraglus alpinus* L., *Oxytropis campestris* (L.) Dc., and *O. splendens* Dougl. ex Hook. are reported as larval host plants (Bird *et al.* 1995; Guppy & Shepard 2001). Other Fabaceae are used in Europe and elsewhere (Scott 1986; Guppy & Shepard 2001). *A. alpinus* and *O. splendens* can be found in the meadows we studied. Wyatt (1957) describes nectaring on *Arnica alpina* (L.) Olin near Aklavik in the Northwest Territories and Roland (1982) indicates that *Erigeron aureus* and *Tonestus* (=*Haplopappus*) *lyallii* are preferred nectar flowers near our study site. This species is a meadow resident using plants within some meadows for both larval and adult resources.

Pieris rapae – Larvae of this species feed on many Brassicaceae, as well as on *Raphanus raphanistrum* L. and *Tropaeolum majus* L. (Scott 1986; Bird *et al.* 1995; Guppy & Shepard 2001). *P. rapae* prefer agricultural areas, especially those rich in Brassicaceae crops, particularly cabbage (Bird *et al.* 1995; Guppy & Shepard 2001), but can be found in many open habitats. A wide range of nectar flowers have been reported for this species (Iftner *et al.* 1992). It is likely that the meadows contain both local butterflies using mustards found within the meadows as well as immigrants from outside habitats using the abundant nectar flowers.

TABLE 1. Butterfly species and the number of times they were observed nectar feeding on different species of flowers. All observations were made in meadows along Jumpingpound Ridge during the summers of 2003–2006.

Flower	*Nymphalis milberti*	*Colias christina*	*C. meadii*	*C. nastes*	*C. philodice*	*Pieris rapae*	*Pontia occidentalis*
Achillia millifolium					2	2	20
Agoseris glauca			1	5	6	2	18
Arnica augustifolia					1		
Campanula uniflora				1	1	2	
Castilleja occidentalis							1
Delphinium bicolor						1	1
Epilobium angustifolium				1		1	4
Erigeron caespitosus		2	5	2	4	7	61
Erigeron peregrinus			1		5		3
Gaillardia aristata							3
Gentianella amarella					1		
Hedysarum sulpurescens					1		
Potentilla fruticosa				1	1	3	10
Potentilla gracilis				1			2
Rhinanthus minor							2
Sedum lanceolatum		1		1	1	2	2
Senecio canus	1						1
Senecio lugens							1
Solidago multiradiata	1	2	2	1	1	3	9

Pontia occidentalis – Brassicaceae are the primary larval host plants (Bird *et al.* 1995; Guppy & Shepard 2001). *Chrysothamnus nauseous* (Pallas) Britt. is reported as a nectar source (Opler 1995). At our site *P. occidentalis* is an eruptive species. In most years they are common but not abundant. In 2003 they were extremely numerous. These butterflies use the meadows for nectar flowers and hilltopping when abundant, but there is also likely an endemic fraction using mustards found in the meadows as larval host plants.

Nectar feeding and floral abundance. Observations of butterflies were conducted as part of an on-going mark-recapture study. This study primarily focuses on the spatial population dynamics and effects of rising tree-line for *Parnassius smintheus* Doubleday, but we also observe and conduct mark-capture for the butterflies listed above and a few other species. Results and effects of nectar flowers for *P. smintheus* will be presented in detail elsewhere. Meadows were censused for butterflies 3–5 times each year from 2003–2006. Censusing took place between July 15 and August 25 each year. As a part of normal observations, we recorded the species of flowers on which butterflies were observed. In most instances these are cases of nectar feeding, but occasionally butterflies may simply be alighting on flowers. Each captured butterfly was identified using a unique three-letter code on both hind wings with a permanent felt pen. This method ensured that we were using multiple individuals in our estimates of flower electivity. If the same butterfly was observed feeding within a short period of time, only the first observation was considered.

The abundance of flowers was estimated 1–2 times in each meadow, each year. We counted the number of flowers of all species within a varying number of 2 × 10 m, randomly placed transects. The number of transects per meadow varied to provide approximately proportional coverage. In 2003 and 2004 all flowers in bloom were quantified, while in other years we only quantified flowers used by *Parnassius smintheus* and the other butterflies.

Analyses. To examine nectar flower use we simply tallied the number of times that butterflies of each species were observed feeding on different species of flowers. To examine electivity in nectar flower use, we compared the observed number of feeding events to an expected number, based on the relative abundance of each flower species. The expected number assumed that nectar flowers should be used in proportion to their abundance if there is no electivity. Over-use in comparison to the expected indicates electivity while under-use would indicate repulsion. Statistical tests of observed versus expected nectaring events were based on a χ^2 distribution (Zar 1999). All tests were conducted within meadows and only when flower counts and feeding observations were made within seven days. We also limited analysis to cases where there were five or more independent observations of nectar feeding for each butterfly species and used a significance level of $\alpha = 0.01$, as cases where $N^2/k < 10$ may show bias. To examine finer-scale electivity, we restricted analyses to only those species of flowers on which feeding had been observed during the study. For an occasion where nectar feeding was observed on a species of flower that was not present in any flower survey, we added one

TABLE 2. Electivity among flowering species. A varying number of nectar feeding events (N) were observed for different species in different meadows on different dates. The first test (χ^2 and df on the left) was for electivity among all species in flower. The second test and the preferred species was for electivity only among flowers used (Table 1). Note that degrees of freedom can vary among meadows within dates due to differences in species use (see Methods). Significant values ($P < 0.01$) are shown in bold.

Species	Meadow	Date	N	χ^2	df	χ^2	df	Preferred species
Colias meadii	S	1 Aug. 2003	7	**77.8**	14	**10.9**	2	*Erigeron caespitosus*
C. nastes	Z	4 Aug. 2003	6	**54.9**	11	**22.7**	5	*Agoseris glauca*
C. philodice	L	6 Aug. 2003	6	**32.0**	5	**11.5**	2	*Agoseris glauca*
Pieris rapae	S	1 Aug. 2003	5	**91.0**	14	**51.7**	7	*Delphinium bicolor*°
Pieris rapae	L	6 Aug. 2003	8	**35.0**	9	11.7	6	
Pontia occidentalis	S	1 Aug. 2003	60	**195.0**	19	**66.7**	12	*Erigeron caespitosus*
Pontia occidentalis	Z	4 Aug. 2003	10	**45.8**	11	7.6	5	
Pontia occidentalis	Y	3 Aug. 2003	5	**98.3**	10	**58.3**	6	*Agoseris glauca*
Pontia occidentalis	M	7 Aug. 2003	18	**3117.9**	16	**669.1**	10	*Erigeron caespitosus*
Pontia occidentalis	L	7 Aug. 2003	7	**35.1**	7	**24.1**	5	*Agoseris glauca*

°*Delphinium bicolor* was not observed in the flower surveys in which nectar feeding was observed.

occurrence of this species to the flower abundance counts when calculating expected values.

RESULTS

Over the four years we observed 214 independent nectar feeding events on nineteen species of flowers (Table 1). *Erigeron caespitosus* had largest number of nectaring events, while *Solidago multiradiata* Ait. was used by the greatest number of butterfly species (Table 3). Only two feeding events for *Nymphalis milberti* and five for *Colias christina* were observed. Although there were small differences, overall nectar flower use by the five most frequently observed butterfly species did not differ significantly among the nineteen species of plants ($G = 82.1$, $df = 72$, $P = 0.20$).

Only in 2003 were there sufficient observations to meet our criteria for analysis of electivity. Among all species flowering within meadows, all butterflies showed electivity for nectar flowers (Table 2). When restricted to only those species of flowers on which each species had been observed feeding, there was still a high degree of electivity.

DISCUSSION

The butterflies investigated here nectar on a diversity of flowers, but as a group they showed similar patterns in their use and preference of nectar flowers. Tufted fleabane (*Erigeron caespitosus*) and False dandelion (*Agoseris glauca*) were preferred species in these subalpine meadows. Strong electivity in combination with the wide range of "usable" flowers suggests that nectar resources are not particularly limiting at this site, thus allowing butterflies to be discriminating in their selection of nectar flowers. The result also indicates that there are differences among nectar flowers in characters that are potentially important to the butterflies.

There are many reasons why certain nectar sources may be preferred, ranging from the accessibility and reliability of the source to the quality and quantity of the nectar (Heinrich and Raven 1972, Watt *et al.* 1974). That the butterflies investigated here showed similar electivity suggests that they are responding to the same characters of these flowers. It is interesting that butterflies restricted to these meadows and more generalist species selected similar flowers. Watt *et al.* (1974) found that flowers used by *Colias alexandra* and *C. meadii* in alpine meadows in Colorado shared similar ultraviolet reflectance patterns and generally had dilute nectar containing simple sugars. A comparison of the UV patterns and nectar chemistry of the flowers in the current system will be profitable.

It would be tempting to equate the presence and abundance of *E. caespitosus* and *A. glauca* with high quality meadow habitat for these butterflies. While it is true that butterflies prefer these flowers and their presence would increase habitat quality, they are not ubiquitous or highly abundant at our site (Table 3). Thus, they are a component of habitat quality for adults

TABLE 3. Nectar flower preferences by each butterfly species as determined by the number of feeding observations on each flower in proportion to the number of flowers of each species. Preferred flowers are in bold. Data shown were collected in 2003. The mean density for each flower species is over all meadows and surveys during 2003.

		Butterfly Species				
Flower Species	Density (mean #/20m^2)	*Colias meadii*	*Colias nastes*	*Colias philodice*	*Pieris rapae*	*Pontia occidentalis*
Erigeron caespitosus	11.2	9	2	4	7	61
Agoseris glauca	6.2	1	5	6	2	18
Solidago multiradiata	163.3	2	1	1	3	9
Potentilla fruticosa	20.1		1	1	3	10
Erigeron peregrinus	10.1	1		5		3
Sedum lanceolatum	15.3		1	1	2	2
Potentilla gracilis	114.3		1			2
Senecio lugens	0.5					1
Senecio canus	0.1					1
Arnica angustifolia	27.6			1		
*Delphinium bicolor**	55.7				1	

**Delphinium bicolor* was not observed in the meadow surveys in which nectar feeding was observed.

but not the sole contributor. Other less preferred flowers that are abundant such as alpine goldenrod (*Solidago multiradiata*) likely are necessary to provide sufficient nectar resources. It is also important to note that while nectar flower use was evaluated throughout the flight season and over several years, electivity could only be examined in 2003 between 1 and 7 August. There are phenological changes in the composition and abundance of nectar flowers. Species such as *Dryas* whose flowers are not usually present after late-July, may be important for the earlier emerging adults, such as *N. milberti*.

Nectar-feeding is an important aspect of butterfly ecology. We have shown what flowers are used and preferred by several species inhabiting sub-alpine meadows. It is our hope that further studies such as this will provide information essential for habitat assessment as well as insight into the evolution of nectar plant use.

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TAXONOMIC AND DISTRIBUTIONAL STUDIES ON THREE *LASIOMMATA* WESTWOOD SPECIES RESTRICTED TO NORTH-WEST HIMALAYA (NYMPHALIDAE: SATYRINAE)

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ABSTRACT. Three species of the genus *Lasiommata* Westwood, (*maerula* Felder, *schakra* Kollar, and *menava* Moore collected from North-West Himalaya have been examined in the light of genitalic studies. Illustrations and a new key to the three species are provided.

Additional key words: Genitalia, brachia, angular appendices, signa, genital plate.

Himalaya is the hub of a great many butterfly species occurring in different ranges. Recent field work in North-West Himalaya has led to the collection of three species that have been identified as *Lasiommata maerula* Felder, *schakra* Kollar, and *menava* Moore. Marshall & de Niceville (1883) placed these species in the genus *Amecera* Butler, which, with *Lasiommata* Westwood, was synonymized under *Satyrus* Latreille by Bingham (1905). Without assigning specific reasons, Evans (1932) and Talbot (1947) assigned these species to *Pararge* Hübner, with *Satyrus* Bingham (Not Latreille), *Lopinga* Moore, *Lasiommata* and *Amecera* as its synonyms (Talbot 1947). In a key to European butterfly genera, Higgins (1975) distinguished *Lasiommata* from *Pararge* by the presence of a pyriform antennal club in the former versus slender antennal club in the latter, placing both in the subfamily Pararginae. Based on this character, the three species studied here belong to *Lasiommata*. A key to the species, diagrams of their genitalia and their distributions are presented here.

Key to Species
Lasiommata Westwood
Common name: **The Walls**

Lasiommata Westwood, 1841, In Humphreys & Westwood, Brit. Butt. Transformations [ed. 1]: 65.

Type species. *Papilio megera* Linnaeus

1. Forewing upperside without brand in male; male genitalia with aedeagus comparatively short, with four pairs of spines at posterior end *maerula* Felder

1a. Forewing upperside with brand in male from inner margin to vein M_2; male genitalia with aedeagus relatively long, with spines either in two pairs or absent at posterior end 2

2. Forewing upperside with black ocellus ringed with broad fulvous ring, subapical similar minute ocellus above it wanting; hindwing upperside with marginal and submarginal lines distinct; female with additional fulvous band on inner side; male genitalia with brachia longer than half the length of uncus; female genitalia with lobes of lamella antevaginalis nearly rounded *schakra* Kollar

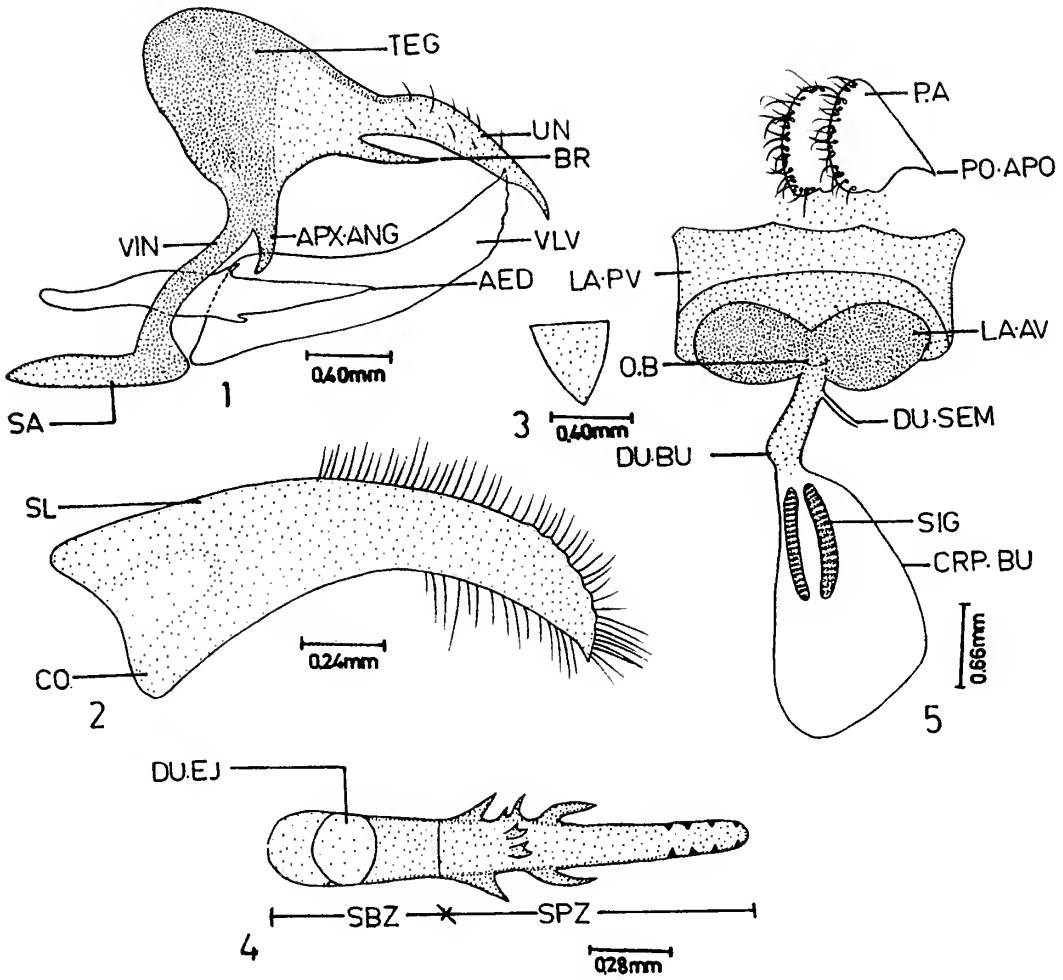
2a. Forewing upperside with black ocellus ringed with ill-defined, faint, yellow ring, a similar but smaller minute subapical ocellus present; hindwing upperside with marginal and submarginal lines missing; female with orange yellow patch between cell and outer margin; male genitalia with brachia nearly1/4 to the length of uncus; female genitalia with central process of lamella antevaginalis more or less triangular *menava* Moore

Genitalic Descriptions
Lasiommata maerula Felder
Common name: **The Scarce Wall**

Felder, 1867, Reise Novara, Lep. 2: 496 (*Lasiommata*)

Male genitalia (Figs. 1–4). Uncus nearly equal to tegumen, weakly curved ventrally, with pointed distal end, sparsely setose dorsally; brachia less than half the length of uncus; tegumen long and broad; appendiees angulares somewhat conical; vinculum shorter than tegumen; saccus moderately long, broader proximally, narrower distally; valva elongated, parallel sided, with pointed apex; juxta more or less triangular; aedeagus short, four pairs of minute, dorsal spines at posterior end, two lateral pairs of spines nearly in the middle, two unequal lateral spines on one side between lateral pairs, a pair of mid-dorsal forked spines, suprazone longer than subzone, ductus ejaculatorius entering dorsad.

Female genitalia (Fig. 5). Corpus bursae elongated, membranous; signa moderately long, paired, parallel, situated longitudinally in the posterior half of corpus bursae, beset with minute teeth; ductus bursae small, moderately sclerotized; ductus seminalis entering ductus bursae near ostium bursae; lamella antevaginalis with two oval, lobe-like structures; lamella postvaginalis quadrangular plate-like; apophysis anterioris wanting, apophysis posterioris small, membranous; papilla analis oblong, pilose.



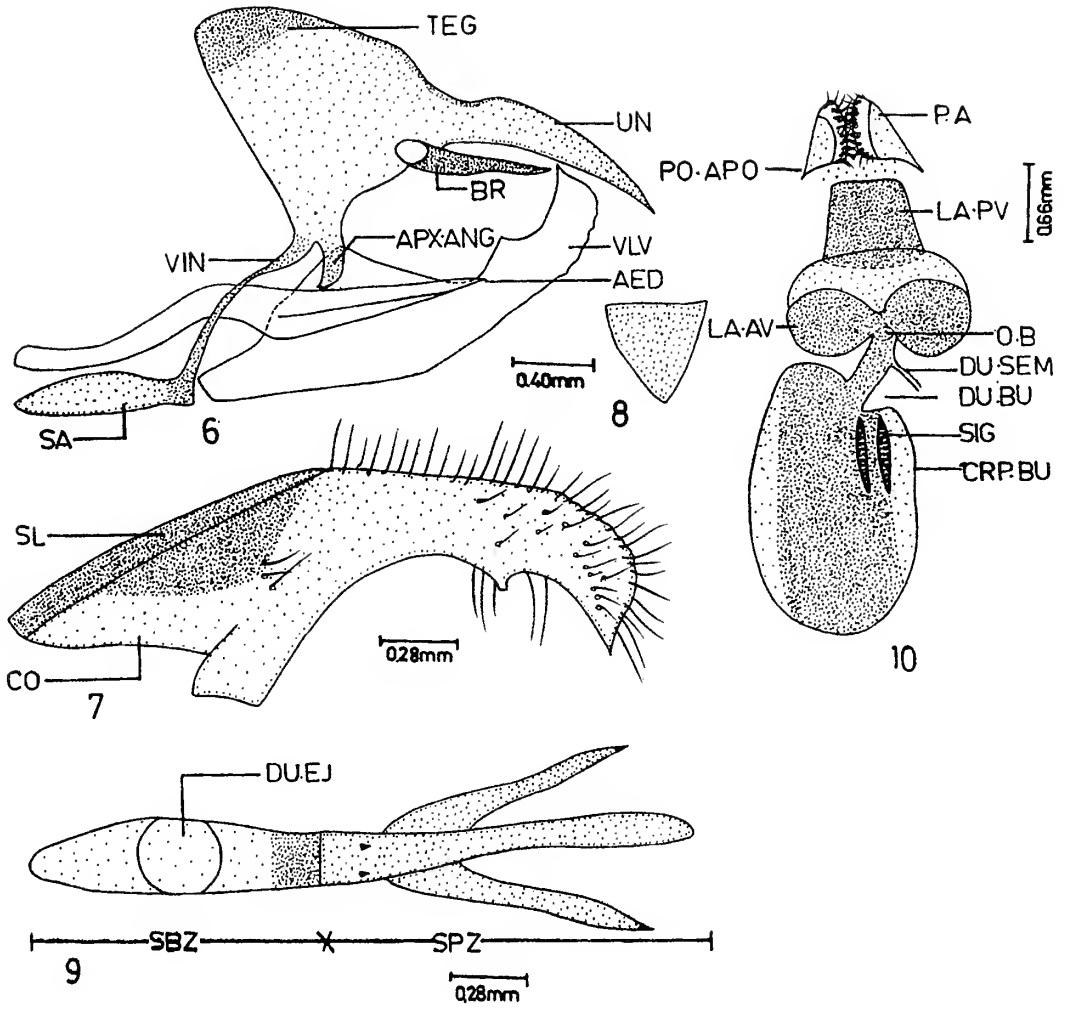
FIGS. 1-5. *Lasiommata maerula maerula* Felder: **1.** Male genitalia (lateral view). **2.** Valva (inner view). **3.** Juxta. **4.** Aedeagus (dorsal view). **5.** Female genitalia (Ventral view). (AED: Aedeagus, APX.ANG: Appendix angularis, BR: Brachium, CO: Costa, CRP.BU: Corpus bursae, DU.BU: Ductus bursae, DU.EJ: Ductus ejaculatorius, DU.SEM: Ductus seminalis, O.B: Ostium Bursae, P.A: Papilla analis, PO.APO: Apophysis posterioris, SA: Saccus, SBZ: Subzonal portion of aedeagus, SIG: Signum, SL: Sacculus, SPZ: Suprazonal portion of aedeagus, TEG: Tegumen, Un: Uncus, VIN: Vinculum, VLV: Valva)

Length of forewing. Male: 27.0–28.0 mm (n=5); Female: 28.0 mm (n=2).

Material examined. Himachal Pradesh: 1♂, 17.ix.92, Sangla, 2680 m, Kinnaur; 1♂, 18.ix.92, Nichar, 2380 m, Kinnaur; 1♂, 18.vii.92, Purthi, 2650 m, Pangi, Chamba. Uttarakhand: 2♂, 29.iv.92, Tiffon Top, 2400 ♀, Nainital; 2m, 28.iv.92, China Peak, 2350 m, Nainital.

Remarks. Relevant literature and present sampling shows that *Lasiommata schakra* (Kollar) is a highly variable species as mentioned under the remarks. Besides making a mention that *maerula* Felder is apparently very rare, Marshall & de Niceville (1883) stated that "probably *maerula* is only a casual variety of *schakra* Kollar, but we retain it as a distinct species, pending further investigations and in deference to Dr. Felder's high authority".

The present field work shows that the species under reference is definitely rare and its males and females have been found to fly together with *L. schakra* in the Kumaon Himalayas (Nainital). The males of *maerula* lack forewing brand and also differ from *schakra* in their marginal and submarginal lines on the upperside of the hindwings. The female is, however, more similar to females of *schakra* and reliable discrimination is possible only through examination of the genitalia. They differ in structures such as the signum, genital plate, ductus bursae and papilla analis. The subspecies is represented by its nominotype.



FIGS. 6-10. *Lasiommata schakra schakra* (Kollar): **6.** Male genitalia (lateral view). **7.** Valva (inner view). **8.** Juxta. **9.** Aedeagus (dorsal view). **10.** Female genitalia (Ventral view). (AED: Aedeagus, APX.ANG: Appendix angularis, BR: Brachium, CO: Costa, CRP.BU: Corpus bursae, DU.BU: Ductus bursae, DU.EJ: Ductus ejaculatorius, DU.SEM: Ductus seminalis, O.B: Ostium Bursae, P.A: Papilla analis, PO.APO: Apophysis posterioris, SA: Saccus, SBZ: Subzonal portion of aedeagus, SIG: Signum, SL: Sacculus, SPZ: Suprazonal portion of aedeagus, TEG: Tegumen, Un: Uncus, VIN: Vinculum, VLV: Valva)

Lasiommata schakra (Kollar)

Common name: **The Common Wall**

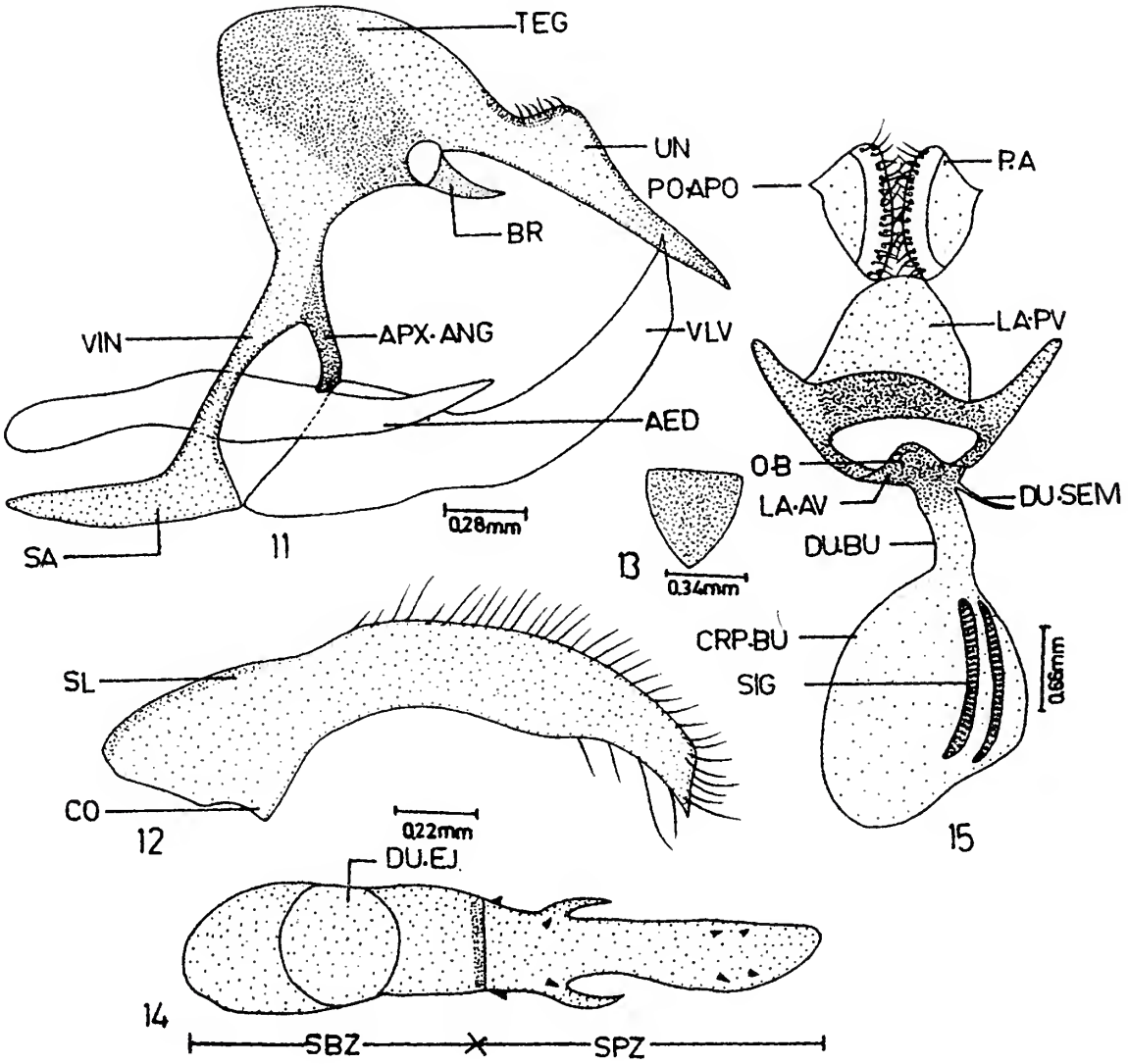
Kollar, 1844, In Hugel's Kashmir (4) 2: 446 (*Satyrus*).

Male genitalia (Figs. 6–9). Uncus slightly shorter than tegumen, broad at base, tapering posteriorly to pointed tip, notch present dorsally between uncus and tegumen; brachia longer than half the length of uncus, distal end pointed; tegumen broad; appendices angulares beak-shaped; vinculum smaller than tegumen, thin; saccus moderately long, narrow proximally and distally; valva elongate, almost parallel sided, with pointed apex, costa with broad, small process, sacculus long and narrow, dorsal margin with small process near distal end; juxta somewhat triangular; aedeagus long, tubular, with two long, lateral processes with pointed ends, two small mid dorsal spines present, subzone smaller than suprazone, ductus ejaculatorius entering dorsad.

Female genitalia (Fig. 10). Corpus bursae globular, well sclerotized in the middle; signa represented by two moderately long, scobinate patches situated longitudinally in the posterior half of corpus bursae; ductus bursae short; ductus seminalis attaching to ductus bursae near ostium bursae; lamella antevaginalis with two lateral, oval structures, below which lies a circular plate; lamella postvaginalis somewhat rectangular plate-like; apophysis anterioris wanting, apophysis posterioris small, membranous; papilla analis oblong, pilose.

Length of forewing. Male: 26.0–27.0 mm (n=96); Female: 25.0–29.0 mm (n=46).

Material examined. Himachal Pradesh: 7♂, 7♀, 28.vi.92, 2♂, 3♀, 3.vii.93, Dal Lake, 1790 m, Dharmsala, Kangra; 2♂, 26.iv.92, 4♂, 3♀, 29.vi.92, 1♂, 4.vii.93, Mcleodganj, 1768 m, Dharmsala, Kangra; 1♀, 30.vi.92, Bhagsunag, 1768 m, Dharmsala, Kangra; 8♂, 6♀,13.ix.92, Chowai, 1800 m, Kullu; 1♀, 20.vi.93, Manikaran, 1737 m, Kullu; 1♂, 18.vi.93, Palchan, 2050 m, Kullu; 2♀, 22.vi.93, Ooch, 1737 m, Manikaran, Kullu; 2♂, 27.vii.92, Kothi-Palchan, 2530 m, Kullu; 1♂, 19.vii.92, Purthi-Kellar, 2650 m, Pangi, Chamba; 1♂, 20.vii.92, Kellar, 2750 m, Pangi, Chamba; 3♂, 2♀, 19.vi.93, Tissa Bridge, 1750 m, Tissa, Chamba; 1♂, 30.iv.96, Katarwai, 1750 m, Tissa, Chamba; 1♂, 1♀,



FIGS. 11-15. *Lasiommata menava menava* Moore: **11.** Male genitalia (lateral view). **12.** Valva (inner view). **13.** Juxta. **14.** Aedeagus (dorsal view). **15.** Female genitalia (Ventral view). (AED: Aedeagus, APX.ANG: Appendix angularis, BR: Brachium, CO: Costa, CRP.BU: Corpus bursae, DU.BU: Ductus bursae, DU.EJ: Ductus ejaculatorius, DU.SEM: Ductus seminalis, O.B: Ostium Bursae, P.A: Papilla analis, PO.APO: Apophysis posterioris, SA: Saccus, SBZ: Subzonal portion of aedeagus, SIG: Signum, SL: Sacculus, SPZ: Suprazonal portion of aedeagus, TEG: Tegumen, Un: Uncus, VIN: Vinculum, VLV: Valva)

16.vi.93, Bharmani Nullah, 2195 m, Bharmour, Chamba; 1♀, 25.iv.96, Bakrota, 2039 m, Dalhousie, Chamba; 4♂,19.ix.92, 1♂, 23.vi.95, 4♂, 1♀, 8.vi.96, Kalpa, 2400 m, Kinnaur; 7♂, 15.ix.92, Bhabhanagar, 1381 m, Kinnaur; 1♀, 18.ix,91, Shimla, 2400 m; 1♀, 14.vi.92, Mahog, 2150 m, Chail, Shimla; 1♂, 2♀, 8.ix.92, Kumarsain, 1485 m, Shimla; 3♂, 25.vi.96, Koti, 2150 m, Chail, Shimla; 7♂, 1♀, 12.ix.92, Taklech, 1600 m, Rampur, Shimla; 4♂, 2♀, 28.v.92, Chambaghat, 1440 m, Solan; 6♂, 1♀, 29.x.92, Kasauli, 1731 m, Solan. Uttarakhand: 2♂, 1♀, 2.vi.93, Bhilaru Pumping Station, 1710 m, Mussoorie, Dehradun; 1♂, 3♀, 7.vi.93, Spring Road, 2005 m, Mussoorie, Dehradun; 1♀, 5.vi.93, Murray Electric Pumping Station, 1645 m, Mussoorie, Dehradun; 1♀, 8.vi.93, Dhanolti, 2250 m, Dehradun; 2♂, 9.vi.93, Bandhal Nadi, 640 m, Dhanolti, Dehradun,; 1♂,1♀, 15.vi.94, 3♂,1♀, 13.vi.95, Chakrata, 2100 m, Dehradun; 8♂, 3.vii.94, Ranikhet,1829 m, Nainital; 2♂, 2♀, 29.iv.92, Tiffon Top, 2400 m, Nainital. Jammu & Kashmir : 5♂, 29.viii.94, Patni Top, 2060 m, Jammu; 1♂, 29,viii.94, Kud, 1700 m, Patni Top, Jammu.

Remarks. *Lasiommata schakra* (Kollar) is one of the most common species collected along the banks of water bodies and stony areas in the localities cited above. Earlier works, such as Marshall & de Niceville (1883), Bingham (1905), Evans (1932), Talbot (1947)

and Wynter-Blyth (1957) did not define clearly the diagnostic characteristics, e.g. subapical ocellus along with other orange spots and post-discal ocelli on the dorsal and ventral sides of the hindwings. The number of orange spots on the dorsal surface of the forewings is always four (including the subapical ocellus), but their size and spacing vary in different individuals from the same as well as different localities. Apart from the subapical ocellus, the remaining three orange spots are well developed in ninety-two individuals and obscure in fifty individuals. The number of post-discal ocelli/spots on upperside of the hindwings varies from three to six. Out of these, three are always black, with white pupils and ochraceous ringed. Talbot (1947) has erroneously indicated that this post-discal row of three to six spots is always black. However, critical examination shows that three black spots are conspicuous in all individuals while fifty-one individuals have one additional yellow spot, thirty-five have two additional yellow spots and five individuals have three additional spots. These additional spots may or may not be black dotted. Individuals collected from the same locality also show variation in the number of these spots (e.g., five individuals with one additional spot and four with two additional spots from Dal Lake, Mcleodganj, Kangra). In view of this variation, six males and four females across its extremes were dissected and found conspecific. The subspecies is represented by its nominotype.

Lasiommata menava Moore

Common name: **The Dark Wall**

Moore, 1865, Proc. Zool. Soc. Lond.: 499 (*Lasiommata*).

Male genitalia (Figs. 11–14). Uncus subequal to tegumen, hump present dorsally at base, distal end pointed, notch present dorsally between uncus and tegumen; brachia short, nearly 1/4 length of uncus, pointed distally; tegumen broad; appendices angulares moderately long, curved inwardly distally; vinculum shorter than tegumen; saccus moderately long, broader proximally, narrower distally; valva elongated, nearly parallel sided, with pointed distal end; juxta more or less triangular; aedeagus long, tubular, proximal half broader, distal half narrow, two lateral moderately long processes, two pairs of minute dorsal spines near posterior end, one pair of minute dorsal spines at the base of lateral processes and another lateral pair of spines behind lateral processes, subzone smaller than suprazone, ductus ejaculatorius entering dorsad.

Female genitalia (Fig. 15). Corpus bursae oblong; signa long, paired, situated longitudinally, with scobinate patches; ductus bursae short and broad; ductus seminalis originates from ductus bursae near ostium bursae; lamella antevaginalis with central process small, somewhat triangular, lateral processes long, joined in the middle; lamella postvaginalis globular, plate-like; apophysis anterioris wanting, apophysis posterioris reduced, membranous; papilla analis ellipsoidal, pilose.

Length of forewing. Male: 25.0–30.0 mm (n=4): Female: 27.0–28.0 mm (n=6).

Material examined. Himachal Pradesh: 1♂, 5♀, 25.vi.95, 2♂, 1♀, 22.vi.96, Hurling, 3150 m, Kaza, Lahoul and Spiti; 1♂, 28.vi.95, Puh, 2700 m, Kinnaur.

Remarks. This species has been reported from many hilly areas, mentioned above (Marshall & de Niceville 1883; Evans 1932; Talbot 1947; Wynter-Blyth 1957). The new specimens, except one, were collected at Hurling along the Spiti River Valley. These localities are new distributional records for this species. One of the males collected from Hurling is different from the remainder as it is much darker in color and possesses only one ocellus in cell Cu1a. However, it is conspecific genitalically. The subspecies is represented by its nominotype.

DISCUSSION

The present study shows that all three species are restricted to North-West Himalaya. Their male and female genitalia suggest that they form a single natural group. The male genitalic structures (e.g. dome shaped tegumen, uncus and distally pointed brachia, almost parallel sided valvae with pointed apex and aedeagus with dorsal teeth) conform to the type-species *megera* Linnaeus of the genus *Lassiomata* Westwood, as has been illustrated by Higgins (1975). The examination of the female genitalia of *maerula*, *schakra* and *menava* shows that paired signa (Pierce & Beirne, 1938) are always present in the corpus bursae. Also the posterior apophyses are reduced and the ductus seminalis always originates from near the ostium bursae.

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EUPHILOTES ANCILLA (LYCAENIDAE) IN THE SPRING MOUNTAINS, NEVADA: MORE THAN ONE SPECIES?

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ABSTRACT. Two independent temporal cohorts of *Euphilotes ancilla* (Lepidoptera: Lycaenidae) with different larval host plants occur sympatrically in portions of the Spring Mountains of southern Nevada. Their diapause intensities (as determined in the laboratory) and flight seasons exhibit little or no overlap, but phenotypes of the cohorts appear identical. It is speculated that they arose from changes in the relative phenology of their larval host plants in response to climatic alterations subsequent to the Pleistocene. Although these *Euphilotes* seem to behave as separate biological entities, their taxonomic level remains equivocal. Until more information is forthcoming, they are recognized as separate subspecies: *E. ancilla purpura* and *E. ancilla cryptica* **n. ssp**.

Additional key words: conservation, new subspecies, Polyommatinae, sympatric divergence.

Euphilotes Mattoni, 1978, a genus of polyommatine Lycaenidae, exhibits an often baffling array of taxa at both specific and infraspecific levels. Not only has their genus-level nomenclature experienced numerous upheavals over the years, but their species-level taxonomy has suffered through chaos dating nearly to their initial discovery. This taxonomy arguably has had less historical consensus than that of any other North American genus of butterflies, with three to as many as eleven species recognized in myriad combinations (Barnes & McDunnough 1917; McDunnough 1938; Mattoni 1954a, 1954b, 1965, 1977, 1988; Downey 1961; dos Passos 1964; Shields 1974; Langston 1975; Miller & Brown 1981; Scott 1986; Pratt 1988, 1994; Shields & Reveal 1988; Pratt & Emmel 1998; Opler & Warren 2002; Warren 2005). This confusion originates from very similar superficial appearances of the numerous taxa; knowledge of larval morphology and host plants, adult genitalia, and geographical and temporal distributions are often necessary for identification.

The life cycles of *Euphilotes* are closely coordinated with those of their larval host plants, *Eriogonum* (Polygonaceae)(Langston 1963; Langston & Comstock 1966; Shields 1975, 1977; Arnold 1983a, b; Pratt & Ballmer 1986, 1993; Arnold & Goins 1987; Pratt 1988, 1994; Mattoni 1990; Peterson 1997). That species-rich genus of plants, including spatially and/or temporally separated varieties, is widespread in and nearly entirely confined to western North America (Reveal 1969, 1978). More than one taxon of *Euphilotes* may co-occur, either synchronously or not, but most co-occurring species use different host plants (Pratt & Ballmer 1986; Ballmer & Pratt 1988; Shields & Reveal 1988). Although there are exceptions, a single taxon of *Euphilotes* uses but one species of larval host plant at any one site (Pratt & Ballmer 1986, Shields & Reveal 1988). Their eclosion is closely consilient with flowering phenology of larval host plants, and nearly all populations are univoltine (Pratt & Ballmer 1986, 1993; but see Newcomer 1964; Langston 1974; Shields 1975, 1977; Pratt & Ballmer 1986; Pratt 1988, 1994; Pratt & Emmel 1998; Davenport 2003). Pupae of some can extend diapause (holdover) through more than one winter (Pratt & Balmer 1986). Phenologies of butterflies may respond to elevational and latitudinal gradients tracking seasonal progression of larval host plants (Peterson 1997; Pratt & Ballmer 1993). Local populations fly for no more than 4–8 weeks annually (e.g., Langston & Comstock 1966; Arnold 1983a, b; Arnold & Goins 1987; Peterson 1995b; Mattoni *et al.* 2001).

The generalities of the life history of *Euphilotes* obscure its complexity wherein members of the genus exploit nearly all possible combinations of spatial, temporal, and larval host plant use patterns (Pratt 1988). These encompass a variety of seasonal, elevational, and

latitudinal replacements, irregular bivoltinism, and co-occurring overlaps in use of larval host plants. Numerous instances exist where two or more taxa are more or less sympatric (either synchronic or allochronic), but these usually use different larval host plants, and are distinguishable morphologically (Pratt & Ballmer 1986, 1993; Pratt 1988, 1994; Pratt & Emmel 1998; fide G. Pratt). Others have spatially approximate and apparently consubspecific populations using different larval host plants that temporally overlap for a minority of their collective flight season (Arnold 1983a). These situations suggest incipient speciation (Arnold 1983a; but see Pratt & Emmel 1998). Genetic exchange was found between phenologically disjoined populations in Washington (Peterson 1995b, 1996). Those populations using the same larval host plant and having overlapping diapause intensities, however, are not sympatric, but elevationally disjunct, and gene flow is thought to be in a stepping-stone fashion along an elevational gradient tracking the phenology of larval host plants (Peterson 1995b).

During more than four decades of investigations of the butterfly fauna of southern Nevada, observations were made on the endemic *Euphilotes ancilla* that occurs as several apparently distinct populations at middle elevations of the Spring Mountains (Clark and Nye counties). This *Euphilotes*, referred to as near both *Euphilotes enoptes enoptes* (Boisduval, 1852) and *Euphilotes ancilla ancilla* (Barnes & McDunnough, 1918), as a subspecies of *E. enoptes*, by Shields (1977), was considered as an undescribed endemic subspecies (Austin & Austin 1980; Austin 1981, 1985). A revision of *Euphilotes* proposed recognition of several species within the *E. enoptes* group, the Spring Mountains' populations became a subspecies of *E. ancilla* (Pratt & Emmel 1998), and this phenotype was subsequently described as *Euphilotes ancilla purpura* by Austin (1998). Its populations are located at the southern extent of the distribution of *E. ancilla* and their flight period extends to the latest reported date for the species.

The first known records of *Euphilotes ancilla* in the Spring Mountains are represented by material at the American Museum of Natural History taken in July 1928. Subsequently, there had been few reports (single records in 1936, 1959, 1966, and 1972) until the late 1970s when it was found to be locally common on occasion, flying from early June to mid-August at elevations between 1860 and 2190m (Austin & Austin 1980). Later, Weiss *et al.* (1997) had records for 11 sites between 1800 and 2500m with an overall flight season from mid-May through mid-August. The majority of records was from early June to early August with no notable peak.

The tendency of males of *Euphilotes ancilla* in the Spring Mountains to congregate on stream banks and at seeps from late May to mid-June undoubtedly biased early accounts of distribution. The known and assumed only larval host plant (and principal adult nectar source), *Eriogonum umbellatum* Torrey var. *subaridum* S. Stokes, is widespread in these mountains, but is often locally sparse. No butterflies were found at stands of this plant during May and June, since flowers had yet to appear. Prior to 1998, females had not been found until late July when the then known host plant came into bloom. These records unfoundedly suggested that males emerged a month or more before females and often occurred at mud in large numbers early in their flight season. Males were infrequently seen at mud after late June, although this resource is continually available.

Original observations on phenology of *Euphilotes ancilla* in the Spring Mountains were paradoxical for several reasons. *Euphilotes* was not known to emerge several weeks before host plants reach early bloom (Langston 1963; Pratt & Ballmer 1986). Extreme protandry was unknown among *Euphilotes*; the lag of female emergence had not been found to exceed eight days (Arnold 1983a; Peterson 1995b). Males of a short-lived butterfly with residence times of two to nine days (Arnold 1983a, b) would not be expected to eclose more than a month before the first females emerge. It was fortuitously discovered during 1999 that two varieties of *Eriogonum umbellatum* serve as larval host plants for *Euphilotes* in the Spring Mountains: an early-flowering *Eriogonum umbellatum* Torrey var. *juniporinum* Reveal and a late-flowering *Eriogonum umbellatum* var. *subaridum*. This suggested that perhaps this *Euphilotes* exhibited a simple bivoltine life history with seasonal replacement of larval host plants, a strategy not unusual among multivoltine butterflies. Since, however, bivoltinism and seasonally alternate larval host plants are not common among members of *Euphilotes*, investigations reported here were focused towards a fuller understanding of the distribution and biology of these butterflies.

MATERIALS AND METHODS

Distribution and phenology. Spatial and temporal distributions of *Euphilotes ancilla* were determined from specimens, published accounts, field notes, and more recent surveys. These latter were facilitated by historical records of and searches for larval host plants, and observations at water sources where males are encountered at mud. Surveys along roads and trails in the Spring Mountains were undertaken from late April through September 1998–2003 including the west slope

of the range from Wheeler Pass southeastward to Potosi Mountain and the Red Rock area and the east slope from Big Timber Spring to Harris Mountain (Fig. 1).

To further quantify phenology of *Euphilotes ancilla* and its larval host plants, five transects were established near Willow and Cold creeks during spring 2002. Three were within stands of *Eriogonum umbellatum* var. *juniporinum* (two on a hillside above Willow Creek, 1825m and 1850m in elevation, and one on a flat along the road from Willow Creek to Cold Creek, 1775m) and two in stands of *E. umbellatum* var. *subaridum* (one at Cold Creek, 1825m, and the other adjacent to a seep between Willow and Cold creeks, 1775m). These transects were walked at 7–13 day intervals during 2002 and 8–11 day intervals during 2004 encompassing nearly the entire flowering season of *Eriogonum*. Stage of flower development was recorded for the first 100 plants encountered as none, early bud, late bud, flower (at least one per inflorescence), and senescent (e.g., see Peterson 1995b). The proportion of plants producing flowers was the maximum in bud or flower, or that had senesced on any one visit (Fig. 2 shows only those that were in flower). The presence of *Euphilotes* was also recorded.

Diapause intensity. Methods for determining intensity of diapause followed those of Pratt & Ballmer (1993). This, the mean number of days between removal from refrigeration and eclosion, is a standard indicator of flight season in *Euphilotes*; its caveats were discussed by Pratt & Balmer (1993). Using these data, the occurrence and intensity of diapause in different populations may be compared. If two populations are distinct, they will have different emergence patterns that, in the field, should correlate with flowering phenologies of their respective larval host plants.

Larvae of *Euphilotes ancilla* were obtained by examining larval host plants, with special attention to parts of plants with ants (see also Arnold 1983a). These parts and those on which larvae were found were clipped and transported in plastic containers to the laboratory in Henderson, Nevada. In the Willow and Cold creeks area (1775–1825m in elevation; hereafter referred to as Willow Creek), 86 larvae were collected between 3 and 23 June 2000 from *Eriogonum umbellatum* var. *juniporinum*; eight were collected in early August 2000, and 31 were collected between 3 and 22 August 2001 from *E. umbellatum* var. *subaridum*. Searches for larvae elsewhere in the Spring Mountains proved fruitless during 2000 and 2001, since host plants had apparently been negatively impacted by continuing drought.

Once at the laboratory, larvae from all samples were individually separated into small plastic cups covered with elastic nylon. Flowers of appropriate host plants were maintained in each cup in a plastic bud vial provided with water that kept flowers fresh and potentially more hydrated than under field conditions. Larvae were kept at room temperature (*ca.* 21°C) and ambient light. Containers and vials were cleaned daily and provided with fresh host plant as needed. Excess host plant was refrigerated at 4°C and replaced by new stock from the field every 3–5 days. Larvae were so maintained until they pupated. Pupae were placed on a bed of sterilized crushed limestone (collected from the same location as the larvae) in a ventilated plastic container, separated by date of pupation, and stored at room temperature and light regimens. On 1 October of each year, all pupae were refrigerated at 4°C. Their container was covered with paper towels (not touching the pupae) that were lightly misted every 7–10 days to prevent desiccation. Pupae were removed from the refrigerator the following 1 February and maintained again at a room temperature of about 21°C. These were monitored daily until pupae eclosed, died, or failed to break diapause. These latter were then again subjected to the refrigeration protocol as above.

Results and Discussion

General biology. Larvae of *Euphilotes* feed on reproductive parts of *Eriogonum*, including sepals, flowers, pollen, and young seeds (Arnold 1983a; Pratt 1988, 1994; Mattoni 1990; Pratt & Ballmer 1993; Peterson 1997). Those from the Spring Mountains are typical, feeding largely on developing fruit, although one was recorded feeding on pollen. They remained concealed within inflorescences throughout development. No larval nests were constructed, although they are in some populations of *Euphilotes* (Pratt & Ballmer 1986). Pupation by *Euphilotes* is usually in the soil or among debris at the base of the larval host plant, but may occur in flower heads or near bases of leaf axils (Arnold 1983a, Arnold & Goins 1987). Since all pupae in this study were on the floors of larval containers, these populations are assumed to pupate in litter or soil.

Many larvae in third and fourth instars during 2000 were attended by ants. These attendant ants included five species, four associated with larvae from the first flight and two with the second; one of these occurred during both flights (Table 1). Data for associations of ants for the second flight may have been biased by the few larvae encountered. In contrast, no ants were seen attending larvae during 2001. All species of ants were, as expected, those of the secretion-nectar feeding guild (e.g., Hölldobler & Wilson 1990). Associations of ants with larval *Euphilotes* are facultative and seemingly

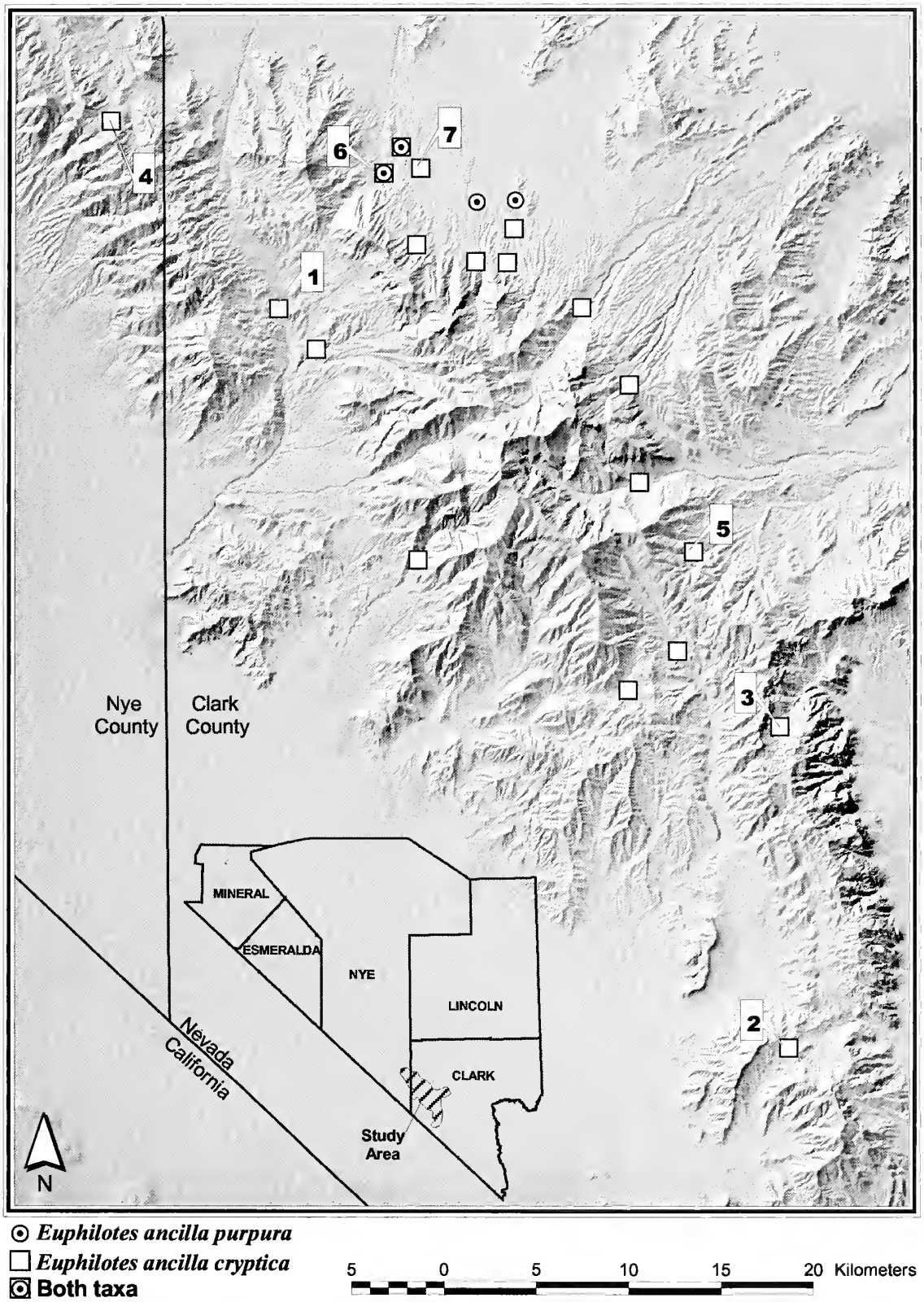

FIG. 1. Distributions of *Euphilotes ancilla* in the Spring Mountains, Nevada. Identified sites are (1) Wheeler Pass, (2) Potosi Mountain, (3) Switchback Spring, (4) Big Timber Spring, (5) Harris Mountain Road, (6) Willow Creek, and (7) Cold Creek.

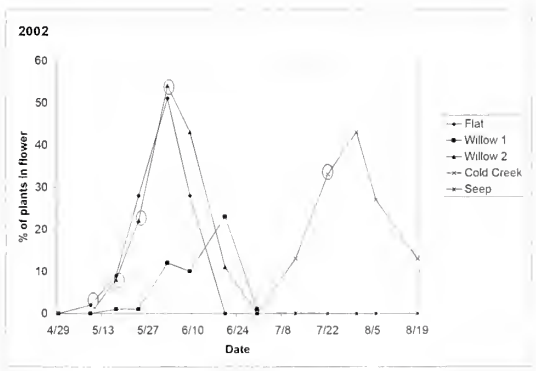

FIG. 2a. Phenology of *Eriogonum umbellatum* near Willow Creek, Spring Mountains, Nevada during 2002 (encircled datapoints indicate presence of adult *Euphilotes*; see text for locations of transects).

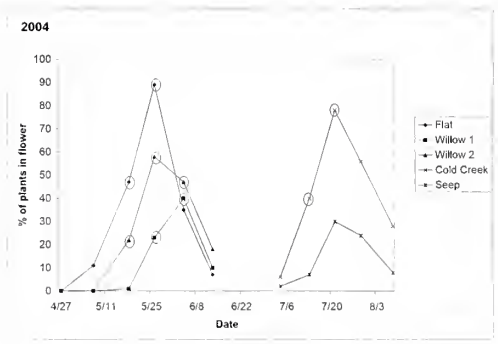

FIG. 2b. Phenology of *Eriogonum umbellatum* near Willow Creek, Spring Mountains, Nevada during 2004 (encircled datapoints indicate presence of adult *Euphilotes*; see text for locations of transects).

unpredictable (Ballmer & Pratt 1991, Peterson 1995a; see also Shields 1973); it is therefore not surprising that none was found during 2001. These data on attendance by ants, although likely incomplete, represent the first reports for these populations.

Of the 86 larvae collected in June 2000, 81 pupated between 8 June and 1 July, the eight collected in August 2000 pupated between 14 and 24 August, and 30 of the 31 collected in August 2001 pupated between 11 August and 3 September. Larvae from *Eriogonum umbellatum* var. *juniporinum* pupated over a period of 24 days at an average of 12.8 days (SEM = + 0.55, variance = 38.6%) after the first pupation; those on *E. umbellatum* var. *subaridum* also pupated over a 24 day period at an average of 16.6 days (SEM = ±0.84, variance 6.3%). These means are significantly different (t = 1.982, df=109).

Of the 125 immatures collected, one died in the larval stage and five died as pupae, all of unknown causes, and five larvae were intentionally sacrificed for preservation. None was parasitized. The absence of parasitism was unexpected, although Shields (1973) also reported no instances of parasitism. High incidences of parasitism by tachnids (Diptera)(42–60%) and braconids (Hymenoptera)(20%), however, were recorded among *Euphilotes* in California (Arnold 1983a; Mattoni 1990); likewise, parasitism by Hymenoptera and Diptera approaching 60% occurred in Washington (Peterson 1997).

The reared sample from the Spring Mountains was female biased (43:65, 39.8% males), although this is not a significant deviation from equality (chi square = 1.871). In two species of *Euphilotes* reared from Californian populations, the sex ratio was nearly 1:1 with males slightly outnumbering females (52.3%; Arnold 1983a); Shields (1975) also found equal numbers of males and females.

Distribution and phenology of larval host plants. *Eriogonum umbellatum* var. *juniporinum* was encountered in the Spring Mountains only in the northeastern portion of the range at elevations of 1775 to 1950m. At these sites, its dispersion is patchy on dry slopes in sparse piñon-juniper woodland and in areas of disturbance (especially old burns), with loose soils of high limestone content. It blooms from late April to late June. This phenotype of *Eriogonum umbellatum*, with cream-colored flowers and a rather prostrate growth form, was described relatively recently and reported from White Pine and Lincoln counties of Nevada (Reveal 1985a, b). In the Spring Mountains, this is apparently the plant previously identified as *Eriogonum umbellatum* var. *versicolor* S. Stokes (Beatley 1976; Kartesz 1987). That plant has also been recorded in upper Clark Canyon (Beatley 1976), but that record was

TABLE 1. Ants associated with larvae of *Euphilotes ancilla* in the Spring Mountains, Nevada during 2000.

Species	Number	Date
MYRMICINAE		
Crematogaster mormonum Emery	14	3, 8, 9, 17 June; 3 August
Monomorium minimum (Buckley)	9	3 August
DOLICHODERINAE		
Forelius pruinosum (Roger)	3	3, 8, 9 June
FORMICIMAE		
Camponotus hyatti Emery	13	3, 8, 9 June
Formica laeviceps Creighton	6	3, 8, 9 June

not reverified since parts of Clark Canyon are privately owned and inaccessible. Most *Eriogonum umbellatum* var. *juniporinum* had one to several flower heads during 2000. It was in flower (10–20% of the plants) on the first visit to Willow Creek on 20 May, with bloom continuing through 28 June; these largely appeared to produce seeds. During 2001, the majority of plants again had one to several flower heads and also largely appeared to produce seeds. The flowering season extended from 9 May through 30 June 2002 (Fig. 2a); senescence was rapid after mid-June. Plants at two of the three transect sites flowered more or less synchronously peaking in early June; those at the remaining site exhibited a peak in mid-June (Fig. 2a). The proportion of plants producing flowers differed between sites with maxima of 38 to 64%. In 2004, the plants were in flower from 7 May to after 13 June. Those at two sites again peaked simultaneously, but in late May, and the other peaked in early June (Fig. 2b). The proportion of plants that produced flowers (63–90%) exceeded that in 2002.

The distribution of the more apparent, brightly yellow-flowered, and erect *Eriogonum umbellatum* var. *subaridum* in the Spring Mountains has been better documented both historically (Clokey 1951; Beatley 1976) and through more recent surveys. It occurs as scattered populations across much of the range on both slopes between about 1800 and 3000m and flowers from July through September. Throughout the Spring Mountains, *Eriogonum umbellatum* var. *subaridum* had a poor flowering year in 2002 with only 5–10% producing flowers, these mostly in shaded situations. Many of the flowers dried before they produced seeds and, at Willow Creek, were heavily grazed by ungulates, severely reducing the number of flowers available to any *Euphilotes* present. It was first seen in bloom in early July and had essentially senesced by the end of August. The plant flowered profusely in 2004 when a large percentage produced flowers and seeds. It was first seen in bloom during early July and flowered at some sites into early September. On two transects studied in 2002, the plant exhibited distinctly contrasting flowering patterns. No plants produced inflorescences at one site, while 58% of those at the other did. These latter bloomed from mid-July to beyond mid-August, with a peak in late July (Fig. 2a). In 2004, both populations produced flowers (33–86% of the plants) between early July and early August with a peak in late July (Fig. 2b).

Distribution and phenology of *Euphilotes*. Surveys since 1998 indicated a broader spatial distribution of *Euphilotes ancilla* in the Spring Mountains than previously known and clarified knowledge of its temporal distribution. *Euphilotes ancilla* is now known from a number of sites distributed across much of the range on both slopes from Big Timber Spring to Switchback Spring in the Red Rock Canyon area and on Potosi Mountain between 1775 and 2750m (Fig. 1). Its spatial and temporal distributions are a subset of those of *Eriogonum umbellatum*. Since all species of *Euphilotes* fly only during the flowering period of their host plants and do not occur far from them, the perceived distributions of butterfly and plant reflect reality, at least in the more readily accessible portions of the Spring Mountains. No butterflies, however, have been found at numerous other sites that support larval host plants. At some of those localities, *Eriogonum umbellatum* var. *subaridum* seems too sparse to support *Euphilotes*; dense and apparent populations of *Eriogonum* are preferred (Shields & Reveal 1988). Other sites appear suitable and may well support the butterfly, but will require visits over several years to confirm recorded absences (e.g., see Shapiro 2006). Adults in populations of *Euphilotes*, including in the Spring Mountains, appear absent or very rare during dry years suggesting holdover pupae (e.g., Pratt & Balmer 1986; Shields & Reveal 1988). Their absence

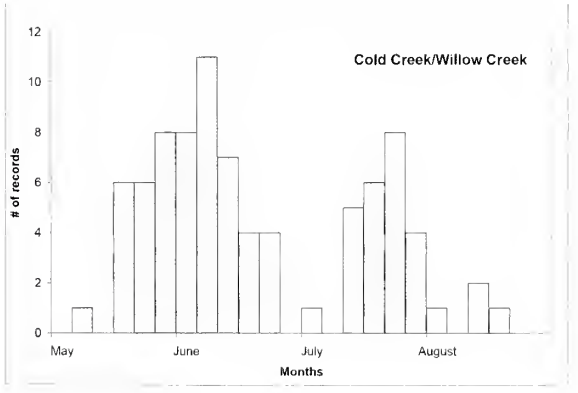

FIG. 3a. Phenology of *Euphilotes ancilla* in the Willow Creek area, Spring Mountains, Nevada.

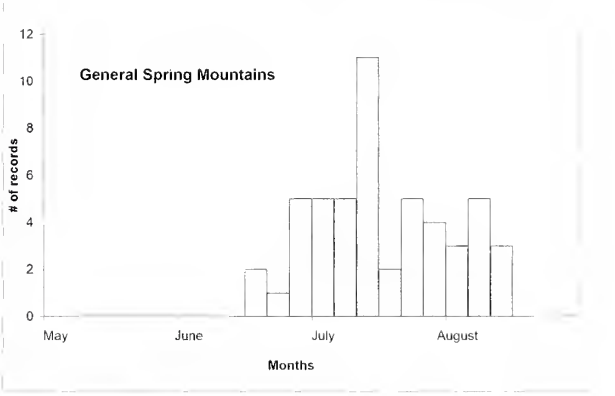

FIG. 3b. Phenology of *Euphilotes ancilla* in the Spring Mountains, Nevada, away from the Willow Creek area.

from a population of *Eriogonum*, therefore, may be more indicative of that year's local weather than the absence of the butterfly. Flowering by *Eriogonum* is related to age of the plant (Arnold & Goins 1987; Arnold 1990) and thus age structure must also be accounted for in considering distributions of *Euphilotes*.

Historical and recent phenological data from Willow Creek reveal two flight periods that could be interpreted as indicating allochronic sympatry. This discovery of two cohorts of *Euphilotes* separated in time and with distinct larval host plants having disparate flowering seasons solved the originally perceived enigma. Cumulative records of adults for this site extend from 9 May to 25 June and from 11 July to 19 August indicating peak flights in early and mid-June and in mid- and late July, with a single record on 3 July (Fig. 3a). These temporal data from a period of 31 years do not account for annual variation in weather, overestimating season length that may occur in any individual year, and underestimating intervals between flight periods. For *Euphilotes*, initiation, peak, and apparent length of flight seasons can vary annually up to about three weeks, but dispersion of emergence times shows little variability (Mattoni *et al.* 2001). In addition, prolonged rainy periods and high soil moisture may extend flowering times of *Eriogonum* and drought may curtail them; both have consequent impact on the eclosion of *Euphilotes* (Pratt & Ballmer 1986; Shields & Reveal 1988; see also Langston 1974). The virtual absence of records at Willow Creek during late June and early July (the three records between 25 June and 11 July were in one year, 1995) is clarified when data from individual years are considered when the two flight periods are separated by more than four to perhaps as many as seven weeks. Thus, no *Euphilotes* were seen at Willow Creek for 31 days between 24 June and 25 July 1998, 35 days from 16 June to 21 July 1999, 45 days from 3 June to 18 July 2000, 45 days from 27 May to 11 July 2001, 49 days from 24 May to 12 July 2002, and 46 days between 29 May and 14 July 2003, and 39 days between 4 June and 13 July 2004. Counts of *Euphilotes* along transects during 2002 and 2004 were, at best, marginally successful probably due to an extended drought. In all instances, however, adults were observed during the early or peak stages of flowering when inflorescences were often still largely in bud (Fig. 2, see also Peterson 1997). It is of interest that the early-flying cohort seems more abundant at mud than the late-flying cohort; water sources are often spatially closer to the larval host plant used later in the season than to that used earlier.

Away from Willow Creek, populations of *Euphilotes* associated with *Eriogonum umbellatum* var. *subaridum* appear to be chronically small, and adults are often not detected during some years. Records for these populations extend from 18 June to 14 August and suggest a single flight peaking in early and mid-July that corresponds largely with the late-flying cohort at Willow Creek (Fig. 3b). These records, however, not only span 85 years, but are from a variety of elevations and slope exposures that, when combined, obscure local phenological patterns. Adults were frequently seen during 1998 and 1999 along Harris Mountain Road, in lower Kyle Canyon, and in the vicinity of Deer Creek. Along Harris Mountain Road, few adults (1–4 individuals) were seen between 19 June and 13 July 2000. Neither adults nor larvae were encountered at known sites in lower Kyle Canyon from 12 July to 30 August 2000, or at Deer Creek from 29 June to 13 August. During July 2001, adults were encountered in fair numbers in the Wheeler Pass area, but none was encountered further south in the Spring Mountains.

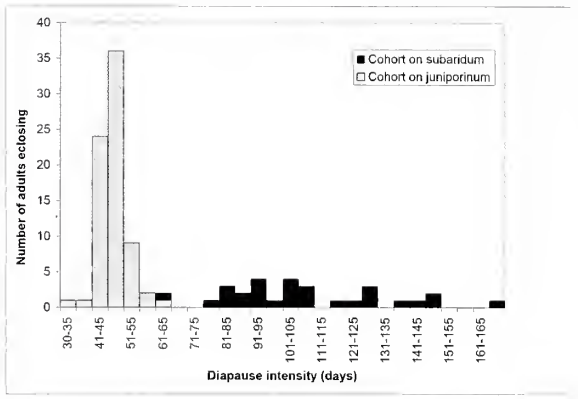

FIG. 4. Diapause intensity of *Euphilotes ancilla* from the Spring Mountains, Nevada.

Diapause intensity. Of the 81 pupae from larvae collected on *Eriogonum umbellatum* var. *juniporinum*, five (6.8%; 1 male, 4 females) emerged without apparent diapause between 26 June and 26 September after an average pupal period of 47.8 days (range 14–106 days, SEM = +18.09). The remaining pupae were refrigerated. One died before and one died during refrigeration. The 74 viable pupae (28 males, 46 females) emerged between 2 March and 6 April 2001 at an average of 46.9 days after removal from refrigeration (range 30–65 days, SEM = +0.54; Fig. 4). Of the eight pupae from larvae on *Eriogonum umbellatum* var. *subaridum* in 2000, seven (4 males, 3 females) emerged between 13 May and 29 June 2001 (one remained as a viable pupa), an average of 116.9 days after removal from refrigeration (range 102–149 days, SEM = +6.25; Fig. 4). Of 31 larvae collected from that plant in 2001,

one was preserved. The remaining larvae pupated and were refrigerated, along with the holdover pupa from 2000. Of these 31 pupae, two died, one disappeared, and 15 from larvae collected in 2001 (7 males, 8 females) eclosed between 3 April and 17 June 2002 at an average of 96.8 days after removal from refrigeration (range 62–136 days, SEM = ± 5.14; Fig. 4); the holdover from 2000, a female, emerged on day 90. The remaining 12 pupae continued in diapause and were again refrigerated for four months. One died, six (3 males, 3 females) eclosed between 28 April and 19 July 2003 at an average of 132.2 days after their return to room temperature (range 87–169, SEM = ±10.58; Fig. 4). The remainder, still viable, was again refrigerated. Two of these died in 2004 after removal from refrigeration and one eclosed after 76 days. The remaining two pupae remain viable through the end of 2005. The eclosion of all 30 pupae from the second flight averaged 109.0 days after removal from refrigeration (SEM = ±4.59). The mean diapause intensities of pupae from the first and second flights and emerging after one year of overwintering were significantly different (t = 19.61, df=87).

Emergence of pupae from the first flight (excluding those that did not enter diapause) extended over 26 days with a variance of 10%, and over 75 days with a significantly different variance of 21% ($F_{15,74}$ = 18.35) for the sample from the late flight collected during 2001 that emerged without holding over (Table 2). The diapause intensities of males and females were identical for first flight individuals, but second flight males preceded females by an average of eight days (combined 2000 and 2001 individuals that emerged the first year after pupation, Table 2). The differences between the sexes in their time of emergence are within the range of reported lag times for *Euphilotes* (Arnold 1983a; Peterson 1995b).

The diapause responses of these *Euphilotes* serve to elaborate the existence of two cohorts. The difference in mean diapause intensity of 62 days is essentially the same as the differences between first dates that adults have been recorded (63 days; 9 May, 11 July) and between the median date that adults of each flight have been seen in the field (55 days; 6 June, 31 July). Also, and perhaps not coincidently, the differences in first emergence dates of the two cohorts from pupae that entered diapause (32 days) and in the median dates of emergence for each cohort (52 days) are nearly encompassed by the number of days the species was not seen at Willow Creek in each of several years (31–49 days; see above). The variance in diapause intensity of individuals of the second flight (21%) was greater than that of the first (10%) and that of holdover pupae was similar, but yet slightly higher (24%). Variance of the emergence dates for the few pupae without apparent diapause was extreme (89%), perhaps suggesting that this is an abnormal response. The lack of diapause is likely an artifact of experimental protocol (*fide* G. Ballmer) and not an indication of bivoltinism.

The seemingly longer flight period of the early-flying cohort may be a function of accumulation of degree days that vary annually and with microhabitats of individual pupae since, under controlled conditions, there was little variance in diapause intensity (82% eclosed within a ten day period); this agrees with a seemingly longer overall flowering period of *Eriogonum umbellatum* var. *juniporinum* (see Table 2). The extended length of the emergence period of the late-flying cohort, with its higher variance, may reflect adaptation to a potentially irregular flowering of the larval host plant due to annual variability in timing and amount of summer precipitation and its effects on soil moisture. Holdover pupae may act as a hedge against drought (e.g., Nakamura & Ae 1977; Waldbauer 1978; Shapiro 1980; Sims 1983). Pratt's (1988) data also indicated a greater spread of emergence dates for *Euphilotes* eclosing late in the season, but perhaps no significant seasonal trend in its variance.

Taxonomic considerations. The revelation of two cohorts of *Euphilotes* in the Spring Mountains spawns uncertainties on their conspecificity. As noted above, nearly all *Euphilotes* are univoltine; bivoltinism occurs in few populations and not in all years (Langston 1974; Pratt & Ballmer 1986). The existence of two ostensibly obligate and site-specific strategies of voltinism within one gene pool seems unlikely. Despite low average vagility of *Euphilotes*, individual dispersal may exceed 1000m (Arnold 1983a; Peterson 1997). Consequently, there is no reason to consider that spatially separated populations in the Spring Mountains exist as closed gene pools (see also Peterson 1995b, 1996). Some other butterflies, however, exhibit life histories with split generations, where some individuals develop directly and others of the same generation enter diapause or develop more slowly, in part as a function of host plant quality or temperature (e.g., Lees & Archer 1980; Wiklund *et al.* 1983; Nylin *et al.* 1989; Nylin 1992; Wedell *et al.* 1997; Schönrogge *et al.* 2000; Fischer & Fiedler 2001). Genetic discontinuity of the Spring Mountains' *Euphilotes* is more likely along a temporal axis.

The origin of and selective agents leading to two cohorts of *Euphilotes ancilla* in the Spring Mountains are at best conjectural. These *Euphilotes* have likely been isolated from populations elsewhere since at least the termination of the Pleistocene as probably have

other taxa of butterflies there (e.g., Emmel & Austin 1998). Their constancy in phenotype and genital morphology across space and time suggests a single origin. During the Pleistocene and perhaps early Holocene, they may have used one or both varieties of *Eriogonum umbellatum*, although earlier flowering taxa seem usual within the *Euphilotes enoptes* species group (Pratt & Ballmer 1986; Pratt & Emmel 1998). These *Eriogonum* likely had overlapping or completely synchronous flowering periods (see arguments by Pratt & Ballmer 1993; Pratt 1994) constrained by a cooler climate (e.g., Spalding & Graumlich 1986; Van Devender *et al.* 1987; Wharton *et al.* 1990). It follows that the butterfly had a single flight throughout the region as is presently usual among *Euphilotes ancilla* elsewhere. With subsequent climatic warming (Van Devender & Spaulding 1979; Van Devender *et al.* 1987), it is feasible that flowering seasons of the two taxa of *Eriogonum* diverged, each blooming at a more favorable season for its respective development (e.g., Pratt & Ballmer 1993; see also Shields & Reveal 1988). This in turn allowed divergence of the butterfly into two cohorts having somewhat different diapause intensities with a selection against genetic configurations outside the optimum imposed by the phenologies of their larval host plants. The warm and dry altithermal (7000–4500 BP)(Antevs 1938, 1948; Baumhoff & Heizer 1965), may well have been the *coup de grâce* for totally allochronic flowering periods of *Eriogonum* and forced the selection for two seasonal cohorts of *Euphilotes*.

The presumptive seasonal isolation of and perhaps absent genetic continuity between these cohorts of

TABLE 2. Comparison of the seasonal cohorts of *Euphilotes ancilla* at Willow and Cold creeks, Spring Mountains, Nevada.

trait	early season cohort	late season cohort
PLANT		
larval host plant	*Eriogonum umbellatum* var. *juniporinum*	*Eriogonum umbellatum* var. *subaridum*
flowering period	late April-late June	mid-July-early September
BUTTERFLY		
flight season[1]	early May-early July	mid-July-mid-August
length of flight season[1]	55 days	39 days
visitation to mud	common to abundant	infrequent
length of pupation period[2]	24 days (n=81)	24 days (n=30)[3]
mean length of pupation period	12.8 days	16.6 days
variance of pupation date	38.6%	6.3%
diapause intensity	46.9 days (range 39-65)	109.0 days (range 62-169)[4]
variance of diapause intensity	9.9%	22.7%
emergence span	26 days (n=74)[5]	75 days (n=15)[6]
mean length of emergence period[7]	16.9 days	35.3 days
emergence time lag (male-female)[8]	-0.3 days (n=28 m, 46 f)	8.0 days (n=11 m, 11 f)
non-diapause pupae	5 (n=81)	0 (n=38)
holdover pupae	0 (n=79)	13 (n=35)

[1] overall from many seasons
[2] time from first to last larva to pupate
[3] from larvae collected in 2001; those collected in 2000 pupated over a 10 day period (n=8)
[4] includes holdover pupae; this was 96.8 days for those emerging the first year after pupation (range 62-149 days, n=22) and 132.2 days after holding over for one year (range 87-169 days, n=6)
[5] only those pupae of larvae collected in 2001 that emerged the first year after pupation; emergence span was 47 days for those collected in 2000 and not holding over (n=7) and 82 days for pupae holding over for one year (n=6)
[6] mean of summation of emergence days after first adult eclosed
[7] non-holdover pupae only
[8] only those pupae that entered diapause; apparent non-diapause pupae had an emergence span of 92 days (n=5)

Euphilotes tempt the consideration of two taxa (the holotype of *E. ancilla purpura* is from the first flight), yet the conundrum of taxonomic level appears. They may be adjudged as host plant and temporal subspecies separated by those and other biological divergences (Table 2, see below). These differences in sympatry, however, are potentially effective isolating mechanisms that could well describe sibling species, despite identical phenotypes. Arguments for two species allied to *E. ancilla* in the Spring Mountains appear most likely (Table 2), yet potential for gene flow exists via the few early flight individuals that may not enter diapause (but see caveat above) and through the overlap (by one individual) in diapause intensity. Low levels of gene flow, however, do not discount species-level differentiation (e.g., Sperling 1993). A continuum of differentiation exists among taxa and, although some may not necessarily possess the range of criteria to consider them full species, they exhibit sufficient differentiation that does not permit inclusion within a single species (e.g., Martin *et al.* 2002). Mallet's (1995, see also Sperling 2003) proposal that evaluation of discontinuities in any of many genetic, ecological, behavioral, and morphological traits to provide useful templates for taxon-level inquiries has merit among these *Euphilotes*. Species-level recognition, however, imposes more questions (e.g., which, if either, is *Euphilotes ancilla*) and requires information on gene flow and genetic distance.

The system, whatever it may be in the Spring Mountains, strongly supports the evolutionary scenario proposed by Pratt & Ballmer (1993) and Pratt (1994) whereby speciation processes in *Euphilotes* are propelled by opportunistic colonizations of alternately available and seasonally disjunct larval host plants concomitant with modification of diapause intensities. These differentiations were probably effected in many instances by climatic perturbations during the Pleistocene and Holocene modifying distributions and phenologies of larval host plants (see also Shields & Reveal 1988; Scriber & Ording 2005). The uses of alternative host plants, often with temporal variables, appear as key events leading to divergence, potentially in sympatry, not only for *Euphilotes* (Pratt 1988, 1994) and other butterflies (e.g., Brown & Heineman 1961; Cardé *et al.* 1970; Pratt & Ballmer 1991; Scott 1998), but also among other insects (e.g., Tauber & Tauber 1978; Smith 1988; Bush 1994; Feder 1998; Abrahamson *et al.* 2001; Emelianov *et al.* 2001; Berlocher & Feder 2002; see also Kankare *et al.* 2005). Differentiation through allochronic isolation may be rapid on the order of a few centuries in some Lepidoptera (Groman & Pellmyr 2000; Thomas *et al.* 2003). The nature of *Eriogonum* facilitates this phenomenon through its species richness and biological diversity, where any one site may be inhabited by several taxa with an array of actual or potential phenological specializations.

As noted above, the use of a seasonal progression of larval host plants is not unusual among multivoltine butterflies. In the Spring Mountains, the two ostensibly phenotypically identical cohorts of *Euphilotes* using different seasonally available larval host plants appear superficially to use a simple and comparatively uninteresting bivoltine strategy. It would, however, be unusual in that *Euphilotes* are not usually bivoltine, and it is apparently unique in that the earlier-flying "generation" does not give rise to the later-flying one. Whatever the level of differentiation between these cohorts, the *Euphilotes* in the Spring Mountains represent a heretofore unknown step within the evolutionary sequence proposed for the genus. The question posed in the title of this paper, however, yet remains with an equivocal answer.

SUBSPECIES DESCRIPTION

Since the two cohorts of the *Euphilotes* in the Spring Mountains are obviously different taxa feeding on different taxa of plants, they at least qualify as host plant races. Only one of these, that flying in May and June (as noted above), has been named. The later-flying cohort is here named and described.

Euphilotes ancilla cryptica Austin & Boyd, new subspecies

Diagnosis. *Euphilotes ancilla cryptica* is distinguished from *E. ancilla purpura* by several biological traits (see above, Table 2) including larvae feeding on *Eriogonum umbellatum* var. *subaridum* (vs. *E. umbellatum* var. *juniporinum*), flight season in July and August (vs. May and June), and diapause intensity of 109 days (vs. 47 days).

Description. Size, wing pattern, and genital morphology apparently identical with *E. ancilla purpura* (see Austin 1998), but with different biological characteristics (Table 2). *Euphilotes ancilla cryptica* is distinguished from other taxa of *E. ancilla* by the same characters as is *E. ancilla purpura* (see Austin 1998).

Types. *Holotype: Male* – NEVADA: Clark Co.; Spring Mts., Cold Creek, 20 July 1978, *leg.* G. T. Austin. Allotype: *Female* – same data as holotype. *Paratypes:* (all NEVADA: Clark Co.; Spring Mountains, *leg.* G. T. Austin, including some paratypes of *E. ancilla purpura*) - same data as holotype (10m, 2f); same location as holotype, 28 July 1977 (5m, 1f), 19 August 1977 (1f); Willow Creek, 20 July 1977 (1 m), 20 July 1978 (7m). Types are all deposited at the McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, University of Florida, Gainesville, Florida.

Type locality. NEVADA: Clark County; Spring Mountains, Cold Creek, 1825m in elevation, towards the northern end of these mountains.

Distribution. The taxon is known from several sites

on both slopes of the Spring Mountains in Nye and Clark counties, Nevada (Fig. 1).

Etymology. This cryptic taxon has been included within *E. ancilla purpura*, since the latter was recognized as different from other phenotypes of the *Euphilotes enoptes* complex.

Discussion. The conspecificity of *Euphilotes* flying early and late in the season in the Spring Mountains is unknown at present, although field and laboratory observations indicate that they are discrete biological entities with little or no overlap of several biological traits. Their sympatry suggests species-level status. Molecular data may yield insights into their relationships, although their divergence may be too recent to give meaningful resolution of their phylogeny and affinities (Peterson 1995b; Nice & Shapiro 1999; Shapiro & Forister 2005). For now, the conservative approach of subspecific-level taxonomy is adopted (see taxonomic considerations above).

Conservation. *Euphilotes ancilla* in the Spring Mountains was considered a species of conservation concern (Anonymous 1998; RECON 2000). With the recognition of two taxonomic entities, management must be focused on each separately. *Euphilotes ancilla purpura* is currently known only from the east slope of the Spring Mountains within relatively small stands of *Eriogonum umbellatum* var. *juniporum* between Willow Creek and West Mud Spring and lower Macks Canyon near the northern end of the Spring Mountains in Clark County (Fig. 1). This taxon, however, is often abundant at those sites. Perhaps its larval host plant is more predictable due to its flowering in the spring when there is potentially more soil moisture than later in the year. The plant's occurrence beneath junipers and pinõns may also provide a moister environment and, along with a relatively unapparent aspect, protect it from grazing.

Euphilotes ancilla cryptica, by contrast, is more widespread as is its larval host plant, *Eriogonum umbellatum* var. *subaridum*. The butterfly occurs in scattered populations from Big Timber Spring to Potosi Mountain (Fig. 1) and in smaller numbers. The host plant sparingly produces flowers in some years, possibly due to drought, and appears subject to greater grazing pressures by ungulates.

The known center of abundance of both taxa of butterflies, in the Cold and Willow creeks area, is faced with considerable disturbance from development and recreation. These, but especially *Euphilotes ancilla purpura*, are at risk with the threat of wildfire exacerbated by invasive weeds and habitat degradation due to unrestricted camping, noncompliant off-road vehicles, equestrian pollution, and feral and introduced ungulates.

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RELIABILITY OF ABDOMINAL PALPATION IN DETERMINING THE MATED STATUS OF OVERWINTERING MONARCH BUTTERFLY (NYMPHALIDAE: DANAINAE) FEMALES IN CALIFORNIA

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ABSTRACT. The abdominal palpation technique is commonly used to evalnate the mated status of monarch females overwintering in both Californian and Mexican winter sites. We found that this method was not reliable in determining the mated status of California overwintering monarch females that had not mated recently. The unreliability of this method was attributed to the reliance of detecting a swollen "pea" (bursa copulatrix) within the female's abdomen by palpation or "feel". The majority of the spermatophores recovered from overwintering females from November to January were too small to cause a significant swelling of the bursa copulatrix that could be accurately and consistently detected by tactile senses. We determined that bursa content weight containing single or multiple spermatophores 10 mg or greater could be detected by palpating the females' abdomen. The palpation method, thus allows quick and easy determination of recently mated females as occurs in February during the mass mating period just prior to the spring migration.

Additional key words: Monarch Butterfly mated status; abdominal palpation; spermatophore

Each fall, monarch butterflies (*Danaus plexippus* L.) migrate to coastal California to winter in groves with specific microclimatic conditions (Leong 1990, 1999; Leong *et al.* 1991, 2004) that will support their winter aggregations. The monarch butterflies begin to arrive in central California by late October and by mid- or late November, winter aggregations ranging from a few hundred to several thousand individuals can be observed on lower branches of wind sheltered inner grove trees (Leong *et al.* 1995, Leong 1999). Overwintering monarch butterflies continue to arrive until late December when their population levels generally reach peak abundance (Leong *et al.* 1995). The population remains at this level until mating activity begins in mid-January (Hill *et al.* 1976; Leong *et al.* 1995). The winter population begins to decline as the butterflies disperse from the winter site to recolonize their spring and summer ranges.

Upon arrival at central California winter sites, > 40% of overwintering butterflies were mated (based on spermatophore counts), and this percentage remained statistically unchanged (Leong *et al.* 1995) through most of the winter season (October to mid-January). This observation conforms with Hill *et al.*'s (1976) findings that mating behavior was infrequent or rare during the winter months and was mainly restricted to few days prior to the spring migration. Tuskes & Brower (1978) reported that mating occurred at a low level throughout the winter, especially during the cluster formation period. They determined the mated status of monarch females by palpating the abdomens with the thumb and forefinger for the presence or absence of a "pea-like" swelling within the abdomen. A female with this swelling was classified as a mated individual while those without were classified as non-mated or virgin females. The "pea" swelling was due to the presence of one or more spermatophores within the female's bursa copulatrix. Van Hook (1999) reported that abdominal palpation of monarchs in the eastern North American population had 95% accuracy for detecting fresh spermatophores and 84% for the detection of extremely deteriorated spermatophores.

This study evaluates the efficacy of the abdominal palpation method in determining the mated or non-mated status of monarch butterfly females overwintering at a central California winter site.

MATERIALS AND METHODS

Winter Site. The Morro Bay Golf Course winter site is located in San Luis Obispo County, California, ≈ 100 m southwest of the 5th putting green (35° 21' 02" latitude; 120° 50' 30" longitude) and the grove is ≈2 km from the ocean. The grove consists of Blue Gum trees (*Eucalyptus globules* Labill.) in the center, while the trees comprising its northern, southern and eastern borders consist of a mixture of old established Monterey pine (*Pinus radiata* Don) and new seedlings of Monterey cypress (*Cupressus macrocarpa* Gord.). Monterey cypress seedlings (now 2 years old) were planted to replace dead or dying Monetery pine trees affected with pine pitch canker. Beneath the Eucalyptus canopy, the understory consists of a mixture of ice plant (*Carpobrotus edulis* (L.) L. Bolus) and poison oak (*Toxicodendron diversilobum* (Torr. & Gray) Greene). The area beneath the grove had a north-south slope of 10°.

Butterfly samples. Butterflies were collected from clusters during the early morning hours (0700–0730 PST), twice monthly (7 and 29 November 1999, 13 and 23 December 1999, 3 and 17 January 2000, and 8 February 2000), using a special (7.2-m telescopic) long-handled net (BioQuip, Gardena, California). Forty females were randomly selected from each sample of netted butterflies and placed individually in numbered envelopes and stored in a cooler for transportation to the laboratory. Within 24 hrs, each butterfly was independently evaluated by the authors and placed into one of two categories using the abdominal palpation method: (1) mated, with a noticeable "pea" and (2) not mated, without a noticeable "pea". The palpation method assumes that the weight or mass of one or more spermatophore within the bursa copulatrix would cause a significant swelling that can be detected tactilely. The same three investigators independently evaluated the biweekly samples of butterflies throughout this study and the order of evaluating the biweekly samples was randomly assigned. After being evaluated, the butterflies were killed by freezing and held in the freezer until they were dissected and examined by the senior author for presence of spermatophores within their bursae copulatrices and spermatozoa within their sperm receptacles.

The bursa copulatrix of each butterfly was carefully dissected for spermatophores. If present, the number and the weight of each spermatophore were recorded to the nearest 0.01 mg using a Mettler-Toledo Balance (AG245). Importantly, the spermatheca (sperm receptacle) of each butterfly was examined for the presence of spermatozoa to confirm a successful mating, as the spermtophore or the neck of the spermatophore was not always recovered from the bursa copulatrix. A spermatheca with spermatozoa appeared opaque white, and the presence of spermatozoa was confirmed by examining the spermatheca contents with a compound microscope using 100X magnification. The spermatozoa appeared as bundles of fine hairs. Each spermatozoan had an oval head with a very long tail.

Statistical Analysis. The data were analyzed using the statistical program Biostat 1 (Pimentel & Smith 1990) for analysis of variance (ANOVA) and Chi-square.

RESULTS

Upon arrival in early November, 16 of 40 females (40%) captured from their winter aggregation were mated (Table 1) based on dissection of their spermatophores and/or the presence of spermatozoa within the sperm receptacle. The proportion of mated

TABLE 1. The average and range of spermatophore weights (mass) of butterflies collected from Morro Bay Golf course winter site during the 1999–2000 winter season. Based on the % mated female (expected) in the monthly samples and the average monthly palpation accuracy scores (observed) of the investigators to predict the mated status of the female, the Chi-square test was not significantly different from a 50-50 determination hypothesis through most of the winter season (November to January) X^2= 5.7; df=5; p>0.05. When the February sample was included in the analysis, the results show a significant deviation from a 50-50 determination; X^2=34.97, df = 6; p= 0.01.

Sample Date	N	Spermatophore weight (mean mg±SD)	Range (mg)	% Mated female	Average Palpation Accuracy scores
7 November	40	1.5±2.6	0 – 8.6	40	49
29 November	40	1.2±2.7	0 – 8.3	40	52
13 December	40	1.7±2.4	0 – 8.6	58	54
23 December	40	2.7±3.3	0 – 11.0	53	53
3 January	40	1.1 ±2.0	0.3 – 6.2	40	60
17 January	40	1.3±3.9	0.1 – 16.7	43	43
8 February	40	20.6±13.2	1.8 – 52.7	98	85

to non-mated females in the biweekly samples remained statistically unchanged (F=0.804, p=0.548, df=5, 234) until 8 February (F=67.33, p=0.001, df=6, 273), when mating activity had increased prior to the spring migration. Our data agree with earlier findings (Leong *et al.* 1995) concerning the seasonal variation of mated females within populations of overwintering butterflies in San Luis Obispo County, California.

The number of correct (hits) and incorrect (misses) assignments based on abdominal palpation of each evaluator was confirmed by dissecting the female. A female was classified as being mated if all or part of the spermatophore was recovered from the bursa copulatrix and/or the spermatheca contained spermatozoa. Notably, the bursae copulatrices of eight females (8 of 280) were void of spermatophores or the necks of spermatophores but were classified as mated because they had spermatozoa within their spermathecae. A female was classified as non-mated if the bursa copulatrix lacked a spermatophore and the spermatheca was void of spermatozoa.

The scores for abdominal palpation ranged from 43% to 60% in accuracy for females collected during most of the winter season (November to January), but increased to 85% in accuracy for females collected in February (Table 1). To test the significance of abdominal palpation accuracy, a Chi-square test was used to determine if our ability to differentiate a mated female from a non-mated female is better than chance (50-50 accuracy determination). The average accuracy scores (observed) of the three investigators were used to predict the actual mated status (expected) of the monthly samples of females, based on the dissection of the bursa copulatrix for the presence (mated) or absence (non-mated) of spermatophores or spermatozoa. The result of the Chi-square test of monthly samples from November to January was not significant from a 50-50 accuracy determination (X^2 = 5.7; df= 5; p> 0.05), suggesting that the abdominal palpation technique was not reliable in differentiating mated from non-mated females during most of the winter season. With the inclusion of the February scores, however, the results revealed a significant deviation from a 50-50 reliability hypothesis (X^2 = 34.97, df= 6, p=0.01) suggesting that recently mated females can be detected with a high degree of accuracy. Ninety-eight percent of the February females were mated and the abdominal palpation technique detected 85% of the recently mated females (Table 1). Those females that were incorrectly misclassified as "virgin" in the February sample had small or flattened spermatophores within their bursae copulatrices.

Since the abdominal palpation method relied on an investigator's ability to feel a "pea-shaped" bursa copulatrix, the data were sorted to eight weight categories along with their corresponding abdominal palpation accuracy scores (Table 2). A series of Chi-square tests were run, starting with the first two categories, progressively adding additional categories until heavier spermatophores caused a deviation from 50-50. The results revealed that the abdominal palpation method was unable to differentiate swellings of the bursa copulatrix containing the spermatophore weights of the first six categories (0.0 mg to 10.0 mg) from those of non-mated females (X^2= 6.79, df= 5, p>0.05). The inclusion of spermatophores weighing greater than 10 mg, however, resulted in better than a coin toss probability (X^2= 23.7, df= 6, p=0.05).

TABLE 2. The average spermatophore weights (mg ±SD) sorted into 8 categories, along with the corresponding sample size and the accuracy scores of the investigators. The "No spermatophores" category also includes highly degraded ones that could not be weighed. To test the 50-50 determination hypothesis, chi-square tests were run on the first two categories, then categories were progressively added until a significant X^2 value resulted. The spermatophore weight category that deviated from the 50-50 determination was >10≤20 mg, which suggests that a bursa copulatrix with spermatophores of this weight would cause a swelling that can be detected by abdominal palpation.

Spermatophore category (mg)	N	Average Spermatophore weights (mg±SD)	Accuracy %
No spermatophores	139	0±0.0	50.4
> 0 ≤2	36	1.2±0.6	37.0
>2 ≤4	36	2.8±0.4	60.2
>4 ≤6	16	4.9±0.6	56.2
>6 ≤8	8	6.9±0.7	70.8
>8 ≤10	8	8.4±0.2	70.8
>10 ≤20	22	15.3±0.1	83.3
>20	15	33.4±13.2	100.0

Spermatophores weighing an average of 15.3 ± 0.1 mg were detected with 83% accuracy, while spermatophores weighing an average of 33.4 ± 13.2 mg had a 100% detection rate (Table 2). With the exception of three females (two from 23 December and one from 17 January), spermatophores weighing >10 mg were all from the February samples.

The percentage of females that were multiply-mated (two or more spermatophores) during the months of November to January ranged from 9.5% to 31.3%. The swelling of the bursae copulatrices of these females could not be differentiated from virgin females because the cumulative weight of their spermatophores was less than 10 mg per multiply-mated female.

DISCUSSION

Based on the accuracy scores, the abdominal palpation method was subjective and only as reliable as a coin toss during most of the overwintering period (November through January). The subjective nature of the method may be attributed to its reliance on tactile senses to differentiate the enlargement of bursae copulatrices of mated females from non-mated females. The weights of spermatophores recovered from overwintering females from 7 November 1999 through 17 January 2000 (n=240) averaged from 1.1 mg to 2.7 mg (range 0 – 16.7 mg). At these weights, the presence of single or multiple spermatorphores within the bursa of mated females did not cause a discernable swelling that could be differentiated tactilely from a non-mated female. Chi-square tests showed that we were not able to accurately detect spermatophores less than 10 mg in mass (Table 2). Van Hook (1999) reported that her use of abdominal palpation was 85% accurate in detecting old spermatophores in 32 females collected in March 1997 from the Sierra Chinqua overwintering site in Michoacan, Mexico. The masses of the spermatophores were not reported. Van Hook (1999) also reported that spermatophores in butterflies in her study deteriorated at a slow rate as compared with rates reported by Oberhauser (1992) for summer breeders. We were able to detect spermatophores with masses between 10 and 20 mg with 83% accuracy (Table 2). The old spermatophores detected by Van Hook could have been within this size range since her March 1997 samples had not yet begun their active migration activities, which could cause a more rapid degradation of the spermatophores.

We were able to accurately detect the larger, fresh spermatophores in recently mated females. Ninety-eight percent of the 7 February 2000 sample were mated and had spermatophores averaging 20.6 mg (range 1.8 – 52.7 mg), 85% of which we accurately detected (Table 1). Spermatophores weighing > 20 mg (ave. 33.4±13.2) were detected 100% of the time (Table 2). Van Hook (1999) reported that abdominal palpation was 95% accurate in detecting fresh spermatophores for butterflies sampled between 15 January and 25 March 1985 from the Sierra Chinqua overwintering site in Michoacan, Mexico. Females surveyed were collected during the mating activity period prior to their spring migration. Like Van Hook, we misclassified five February females (12.5%) as being non-mated. These females contained "old" spermatophores from earlier matings. Monarch butterflies at California overwintering sites may transfer smaller spermatophores (Frey 1999) than butterflies in Mexico and this may account for the lower accuracy in our study.

Oberhauser (1992) estimated that the rate of spermatophore degradation within the bursa copulatrix was 3.3 mg/day. We believe that a spermatophore needs to be a certain critical weight before its presence within the bursa copulatrix can be consistently and accurately felt. Consequently, spermatophores weighing less than 10 mg would have a 50-50 chance of being detected using the abdominal palpation method. The mass of spermatophores recovered from females of the February samples ranged from 11.2 mg to 52.7mg. Using Oberhauser's estimated rate of degradation, the smaller spermatophore would not be reliably detectable after a day and the larger one after ≈ 15 days. Van Hook (1999) found that the rate of degradation was much slower and reported that the spermatophore remained in the freshly mated category for 10 to 11 days. Van Hook, however, hypothesized that the rate of spermatophore degradation would be faster under field conditions where the females are actively flying, feeding, ovipositing and/or migrating to their winter sites.

Since the proportion of mated to non-mated females collected and analyzed during this and an earlier study (Leong *et al.* 1995) remained statistically unchanged during most of the overwintering period, we believe that the majority of the spermatophore contents are broken down and absorbed into the female's system within 15 days of being deposited in the bursa copulatrix. What persists, at least for mated, overwintering females at California winter sites, is the chitinous neck and/or deflated sacs containing remnant amounts of the spermatophore materials. Of the 280 bursae copulatrices examined, 8 had no recoverable neck and/or sac of the spermatophore. The only evidence of mating in these females was the presence of spermatozoa in the their sperm receptacle. All females with "old" spermatophores had spermatozoa in their

sperm receptacles. While Van Hook (1999) did not look for spermatozoa, she noted that 31% of all females in her samples had mated long before the mass mating period in February, which is similar to our findings.

With the exception of three females, the weights of the spermatophores recovered from California overwintering females (November to January) fell below the minimal mass of 10 mg, and it is not surprising that our scores did not differ from 50-50 reliability. Significantly, the three females with spermatophores >10 mg likely were mated during the winter period prior to the mass spring mating period, and comprised just 1% (3 of 240) of the females collected and examined. This low level of mating supports the hypothesis by Hill *et al.* (1976) and observations by Tuskes & Brower (1978) that mating among butterflies in California is rare or infrequent during the winter months and does not significantly influence the proportion of mated to non-mated females within the overwintering population. Tuskes & Brower (1978) reported that 16% of females were mated upon arrival at the winter site but their determination was based on abdominal palpation and was not confirmed by examining the females for spermatophores or for spermatozoa. We believe that their estimate was lower than the actual numbers of mated females because of their inability to differentiate between the bursa swellings of mated females with spermatophores <10 mg from non-mated females. We found that 40% of the females were mated and our results agreed with an earlier study (Leong *et al.* 1995) where 51% of bursae copulatrices of the arriving females had spermatophores.

Van Hook (1999) determined levels of polyandry by counting the number of spermatophore stems extending out of the ostium of the bursa using a dissecting microscope (10–30X). We found examination of the bursa ostium for the presence of the spermatophore (thread) stem impractical and difficult under field conditions using a 10–20X hand lens because it required finding the spermatophore (thread) stem extending out of the bursa ostium in the dark recess of the female's genital orifice. In contrast to the findings of Van Hook (1999), in which the stem extending from the ostium bursa opening was undetected in only 6% of mated females, we were unable to find the stem in more than 20% of confirmed mated females. We therefore conclude that inspection for spermatophore stems without dissection in California overwintering monarchs may greatly underestimate the number of mated females surveyed.

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OLD HICKORY RECORDS AND JOHN ABBOT'S DRAWINGS: NORTH AMERICAN HOST RECORDS EVALUATED FOR *PAPILIO GLAUCUS*

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ABSTRACT. Accurate host plant records are an invaluable tool for anyone conducting research on the Lepidoptera. Recently, Calhoun (2007a, b) presented excellent reviews of two sets of John Abbot's butterfly life history illustrations. Within this review hickory, *Carya* sp., is identified as a host of *Papilio glaucus* (L.) based on Abbot's illustrations and records in the literature. Here we evaluate the potential for hickory, *Carya* spp., to be used as host plants by the tiger swallowtail butterflies, *Papilio glaucus* and *P. canadensis* (R. & J.). Results from larval survival and ovipositional behavior trials indicate that *Carya* spp. should not be considered hosts for these butterflies.

Additional key words: *Papilio*, host plant use, *Carya ovata*, *Carya laciniosa*, polyphagy, Papilionidae.

INTRODUCTION

Fifty-four of John Abbot's life history illustrations were recently carefully evaluated by Calhoun (2007a, b). The services provided by these careful host affiliation analyses are immense for Lepidopterists, and appear accurate and logical. Nonetheless, the accuracy of one of the host plants, 'Hiccory' (*Carya* sp.) of the Juglandaceae, was assumed to have been confirmed as a host (Calhoun 2007a, b), but deserves additional analysis. There is no known convincing experimental evidence or literature verification of hickory as an acceptable/suitable host plant for the eastern tiger swallowtail butterfly, *Papilio glaucus* L. Here we evaluated hickory (2 species) as potential larval food for *P. glaucus* and *P. canadensis* (R. & J.).

The list of host plants putatively used by *P. glaucus* as a species is immense, including 17 plant families, 30 genera, and dozens of species of plants (Scudder 1888–1889; Tietz 1972; Scriber 1973, 1984; Robinson *et al.* 2002; Heppner 2003). However, many of these records are suspected to be incorrect. For example, "hop", *Humulus lupulus*, reported by Scudder (1888–1889) on the authority of J.A. Lintner as a host of *P. glaucus*, may refer to "hop tree", *Ptelea trifoliata*, which is a known host plant of *P. glaucus* (Scriber 1972). Others are a result of successive citation by more recent publications, with mistakes in larval or plant identifications inadvertently passed on as truth (Shields *et al.* 1970; Scriber *et al.* 1975). An example pertinent to the current study, is the record of *Magnolia virginiana* as a host of *Papilio palamedes* reported by Scudder (1888–1889), which is likely derived from a drawing of John Abbot's (see Calhoun 2007a, and cover of The Journal of the Lepidopterists's Society Vol. 61 No. 1). This record was only recently shown to be incorrect (Scriber *et al.* 2000); as remarked by Calhoun (2006), evaluating the validity of host records from John Abbot's drawings has been complicated by the perpetuation of unconfirmed reports in the literature.

Neonate larval stages are especially critical for butterflies (Zalucki *et al.* 2002). In a test of neonate larval survival capabilities of *P. glaucus* and *P. canadensis*, Scriber (1988) evaluated 120 different plant species from 34 different families. In these analyses it was shown that *P. canadensis* could not survive on shagbark hickory, *Carya ovata* (Mill.) K. Koch (Juglandaceae), however, *P. glaucus* was not examined. Here we re-examine potential use of hickory by both butterfly species. The reported use of "Hiccory" in drawings by John Abbot was likely the original source of subsequent *Carya* sp. host plant records for *P. glaucus* reported by Scudder (1872), and subsequently by Couper (1874) and Scudder (1888–89). Scott (1986) reports *Carya* as a host for both *P. glaucus* and *P. canadensis*, but the source of this information is unclear. It therefore seems that all of these reported host records of hickory, *Carya* sp., may be traced directly or indirectly to Abbot's host entry for *P. glaucus* (Calhoun 2007a, b).

The Canadian Forest Service host records for *P. glaucus canadensis* (= *P. canadensis*) during 1947–1955 include 16 species in 6 families, but not hickory, or any other Juglandaceae species (Brower 1958). Extensive efforts to compile the host plant records for *P. glaucus* and *P. canadensis* in New York State (Shapiro 1974; Scriber 1975) and elsewhere (Scriber 1973, 1984) failed to discover any direct observations or literature records of hickory (*Carya* sp.). Recent attempts to rear neonates

of *P. canadensis* on *Carya ovata* (shagbark hickory) resulted in no survival (Hagen 1986; Scriber 1988). Black walnut (*Juglans nigra* L.; also of the Juglandaceae) also failed to support neonate larval feeding and survival of *P. canadensis* (Scriber 1988).

Here we evaluate the acceptability/suitability of two hickory species for neonates of both *P. glaucus* and *P. canadensis*; shagbark, *Carya ovata* (Mill.) K. Koch, and shellbark, *C. laciniosa* (Michx. F.) Loud. We also evaluate the willingness of *P. glaucus* females to oviposit on hickory in a multi-choice arena.

Materials and Methods

Oviposition

Wild caught *P. glaucus* females collected in Oglethorpe County, Georgia (n=18) and St. Joseph County, Indiana (n=4) were shipped to our labs at Michigan State University. *P. canadensis* females were caught and brought directly to our labs from Charlevoix County, Michigan. Ovipositional assays followed similar procedures as those described by Scriber (1993). Females were individually placed into circular oviposition arenas which rotated in front of a 60 watt light bank. Each arena contained leaves from eight different tree species, one of which was the "Hickory" species *C. laciniosa*. The other seven types of leaves were from known host plants of *P. glaucus* and/or *P. canadensis* and included tulip tree (TT= *Liriodendron tulipifera* L., Magnoliaceae), white ash (WA=*Fraxinus americana* L., Oleaceae), black cherry (BC= *Prunus serotina*, Ehrh., Rosaceae), quaking aspen (QA= *Populus tremuloides* Michx., Salicaceae), paper birch (PB= *Betula papyrifera* Marsh, Betulaceae), hop tree, (HT= *Ptelea trifoliata*, L., Rutaceae), and spice bush, (SB= *Lindera benzoin* L. Blume, *Lauraeeae*). The leaves were all of approximately the same size and were spaced at equal distances around the perimeter of the arena.

Butterflies were fed a honey water solution daily and allowed to oviposit until they were too weak to fly (2–5 days). The number of eggs laid on leaves of each plant or non-leaf portions were counted daily and the leaves replaced with new leaves as necessary to insure leaf quality remained high. All eggs laid on each leaf were summed across days and the data interpreted as percentages for each individual female. A small proportion of eggs laid on secondary hosts, non-hosts, and non-plant material such as the plastic sides of the arena or the paper towel lining the bottom are common in ovipositional studies of this nature. Only females that laid more than 20 eggs in the arenas were used in this study (n=7 Georgia, n=1 Indiana).

Larval Rearing

P. glaucus larval abilities were assessed using recently hatched neonates (n=144) from females collected in Oglethorpe County, Georgia (n=10 females) and St. Joseph County, Indiana (n=2 females) placed on leaves of *C. laciniosa* and *C. ovata*. To assess *P. canadensis* larval abilities, neonates from six wild caught females collected in Cheboygan County, Michigan (n=28) were put on leaves of *C. ovata*. As a control, neonates from some of the same families (*P. glaucus* fam.=5, n=39, *P. canadensis* fam.=4, n=29) were also placed on a known host plant of both swallowtail species, black cherry, *Prunus serotina* (Rosaceae) (Scriber 1988), which allowed a comparison of survival and growth rates to those of larvae on hickory. Larval survival on black cherry is known to be comparable to survival on other hosts for *P. glaucus*, including tulip tree, white ash, and hop tree (Scriber 1996). All leaves used in this study came from local areas in Ingham County, Michigan, known to be pesticide free.

The placement of neonates was done using a fine camelhair brush. From one to seven larvae were gently positioned in a paper towel-lined Petri dish along with a *C. laciniosa*, *C. ovata*, or *P. serotina* leaf (in no-choice assays). The leaves were kept moist and turgid by insertion into a water filled aquapic (Scriber 1977). Larval survival was recorded at three and six day intervals. Prior observations by us indicated that in order for a larva to survive beyond three days, it is necessary for it to feed. However, the act of feeding alone does not indicate that a plant is a suitable host for the larvae. Additionally, of those larvae that survive to pupation, we have observed that the vast majority will either have reached the second instar or will be in the act of molting into the second instar by day six.

A comparison of survival on *Carya* sp. versus *P. serotina* was made using Fishers exact test (P-value < 0.05 was considered significant). We combined the two species of hickory for this analysis as no difference was seen in larval survival on the two potential hosts.

Results

The mean number of eggs laid by *P. glaucus* females on *C. laciniosa* was 4.5 ± 2.2 % of the total (Figure 1). Ovipositional preferences were highly variable both among individual females and between females, making firm conclusions difficult. The fact that such a high proportion of eggs were laid on tulip tree and white ash (> 67% of total) may indicate that individual females were exhibiting a high degree of specificity. The proportion of eggs laid on *C. laciniosa* is comparable only to secondary and non-host plants and was never higher than a primary host plant (TT, WA, or HT).

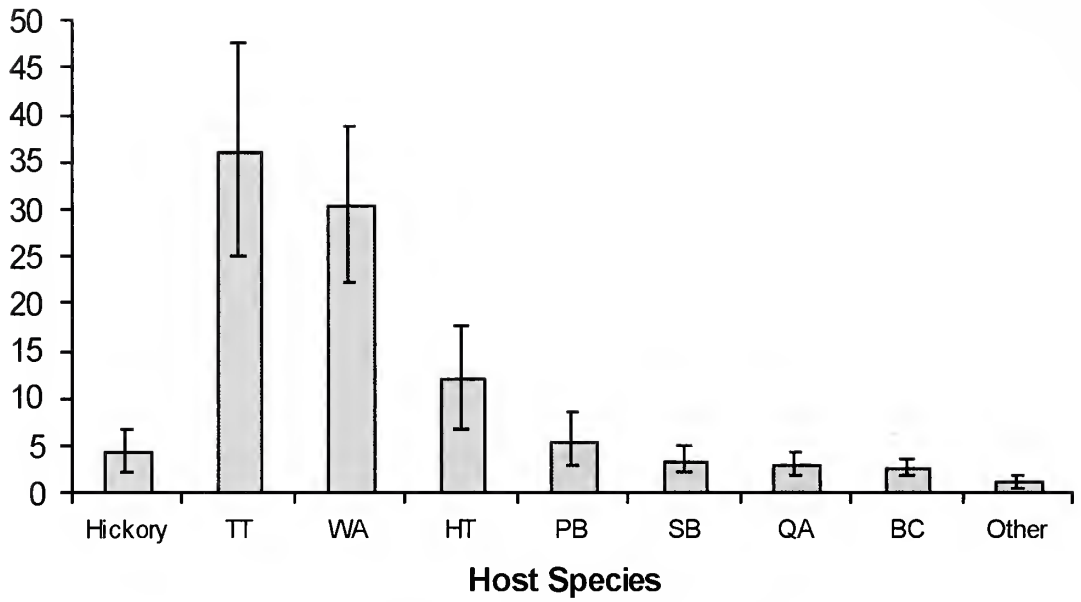

FIG. 1. Mean percent of eggs ± SEM laid by *P. glaucus* females (n=7) on leaves in an eight choice oviposition assay. The plant species are shellbark hickory (hickory), *Carya laciniosa*, tulip tree (TT), *Liriodendron tulipifera*, white ash (WA), *Fraxinus americana*, hop tree (HT), *Ptelea trifoliata*, paper birch (PB), *Betula papyrifera*, spice bush (SB), *Lindera benzoin*, quaking aspen (QA), *Populus tremuloides*, and black cherry (BC), *Prunus serotina*. "Other" indicates eggs laid on either the plastic sides of the oviposition arena or the paper toweling lining the bottom of the arena.

Other ovipositional studies have found similar results for the proportion of eggs laid on non-hosts and marginal hosts in our ovipositional arenas (Mercader & Scriber 2005, 2007). The results from these ovipositional profiles indicate that *Carya* spp. are unlikely to be selected for oviposition as primary hosts, but rare events might be feasible in which eggs are placed on *Carya* spp. when preferred hosts were unavailable or intermingled with leaves of a suitable host (e.g. at the edge of a forest or hedgerow).

In any case, survival by both *P. canadensis* and *P. glaucus* larvae on *Carya* sp. clearly indicate that these plants are unsuitable larval hosts. Only 18 out of 144 *P. glaucus* larvae survived for three days on *Carya* spp., and only one individual lived past six days. This individual died while molting to the second instar on the seventh day. Out of 28 *P. canadensis* larvae, only two lived past the third day and none lived to six days. By comparison, when feeding on black cherry, 27 of 29 individuals of *P. canadensis* survived to the sixth day. In addition, of 39 *P. glaucus* neonates placed on *P. serotina*, 37 individuals survived to day six. Not surprisingly, first instar survival in both *P. glaucus* and *P. canadensis* was significantly different on *Carya* spp. and *P. serotina* (Fishers Exact Test, P< 0.001, Table 1). In this study, individuals placed on *P. serotina* were all from families also tested on *Carya* spp.

DISCUSSION

The reports of larval food plants for herbivorous insects have been frequently constrained in their accuracy by the ability of the observer/reporter to correctly identify the plant and/or the insect. Nowhere are such errors more likely than with polyphagous insects that feed on many species, genera, and families of plants. The eastern tiger swallowtail, *P. glaucus*, and northern (Canadian) tiger swallowtail, *P. canadensis*, are among the most polyphagous of all 560 species of Papilionidae worldwide with respect to larval host plants (Scriber 1973, 1984). Hickory species (*Carya* spp.) are shown to be unsuitable for both species, despite the early references to "Hiccory" by John Abbot for drawings of *P. glaucus* dark morph females (Calhoun

TABLE 1. The number of larvae alive and dead after six days. Differences in survival of *P. glaucus* and *P. canadensis* larvae on *Carya* sp and a known host plant, *P. serotina*, are significant (Fisher's Exact Test, P<0.001).

	P. canadensis		*P. glaucus*	
	Alive	Dead	Alive	Dead
Carya spp.	0	28	1*	143
P. serotina	27	2	37	2

* This individual survived until the sixth day, but died while molting to the second instar on day seven.

2007a, b) and subsequent listings by other lepidopterists. It seems feasible that the compound leaf of an ash species (*Fraxinus* spp.) might have been mistaken by Abbot for the compound leaf of hickory ('Hiccory').

It is not surprising to find additional plant species that may serve as oviposition substrates for these tiger swallowtail butterflies, but which are not acceptable/suitable hosts for the larvae (Brower 1958; Straatman 1962; Wiklund 1975; Berenbaum 1981; Scriber *et al.* 1991; Scriber 1993; Zalucki *et al.* 2002). Experiments with hybrid individuals have shown that the inheritance of traits for larval host suitability and ovipositional preference are independent of each other in several butterflies (e.g. Scriber *et al.* 1991; Forister 2005; Nygren *et al.* 2006; Scriber *et al.* 2008), which may help explain how some of the conflicts between adult host selection and larval host use ability may arise. It is feasible that such willingness to oviposit on "non-hosts" in polyphagous species may facilitate the oscillation between species-wide generalization and local specialization that could help generate new species (Thompson 1998; Nosil 2002; Janz *et al.* 2006). It has been suggested that the transitions from generalist to locally specialized forms may be accomplished by retention of the rank hierarchy but with flexibility in the "specificity" of host use (Courtney *et al.* 1989; Bossart & Scriber 1995; Mercader & Scriber 2005, 2007). Our multiple choice oviposition study, which included *C. laciniosa* as one of eight choices, indicated that a small proportion of eggs may be laid on hickory, but the proportion of eggs laid was very low as observed for other non-hosts (Scriber *et al.* 1991; Mercader & Scriber 2005, 2007). Butterflies in our ovipositional arenas are attracted to the lighted side of the arenas and thus are presented with a different leaf as the arenas rotate, restricting any mechanistic inference (i.e. cues) to contact chemoreception. In the field it is likely that pre-alighting cues (e.g. odor or visual cues) may reduce the encounter rate with *Carya* spp., making the likelihood of these ovipositional 'mistakes' very low.

The absence of survival on *Carya* spp. found in this study for both *P. glaucus* and *P. canadensis* (Table 1) indicates that even if occasional eggs are deposited on *Carya* spp. the neonate larvae are unlikely to survive. The majority of individuals placed on *Carya* spp. did not feed, and the small proportion that did was unable to survive to the second instar. Geographic variation in preference and performance of *P. glaucus* and *P. canadensis* is known (e.g. Scriber *et al.* 1991; Bossart & Scriber 1995), and a few populations of *P. glaucus* may encounter more suitable *Carya* spp. than the ones examined here. However, despite this possibility, our results using two species of *Carya* corroborate previous findings for *P. canadensis* (Scriber 1988) and field observations (Shapiro 1974; Scriber 1973, 1975, 1985) that *Carya* spp. are not host plants of *P. canadensis* or *P. glaucus*. While the services that have been provided by literature reviews are invaluable, the results from this study highlight the importance of verifying host use reports in order not to perpetuate errors in the literature.

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THE HOLY GRAIL OF NEW MEXICO LEPIDOPTERA: SACRAMENTO MOUNTAINS *SPEYERIA NOKOMIS* (W. H. EDWARDS)(NYMPHALIDAE)

Additional key words: extirpated species, Otero Co, Paul Grey, oral tradition

How long does oral tradition persist reliably? I personally am inclined to question anything older than 100 years in the absence of contemporary documentation. To think otherwise endorses claims of living people in non-literate cultures to be 130 years or more of age. Even in America, cults have sprung up that deny the lunar landings and the Holocaust–in the absence of contemporary written records, these denials would become almost impossible to refute by 2060 or 2070.

Let me now examine the case for the existence at some time, past or present, of *Speyeria nokomis* (W. H. Edwards) at a site separated from all other *S. nokomis* by 200 miles, in the Sacramento Mountains of Lincoln and Otero Counties of New Mexico. Such a colony would connect the evolution of the northeast Mexican *S. nokomis wenona* dos Passos & Grey to the mainstream of the species. However,

1. No living person has ever seen a living specimen.
2. No living person has ever seen a museum specimen.
3. No living person knows who took or saw the specimens.
4. No living person has ever seen a first-person written record of a capture or sighting.
5. I know of no pre-1945 (i.e., contemporary) written third-person record of a capture or sighting. (I am disregarding the report of three males from the junction of Hubble and Wills Canyons, near Weed, Otero Co., in 1973, which I am virtually positive is bogus).

Within my lifetime, the credibility of this entity has shifted from the realm of quantifiable doubt to the realm of myth/legend, where myth/legend is defined to be an event devoid of even third-person contemporary documentation. In fact, it is only due to the diligence of Steve Cary (Cary 2003) that I even know of a collector who could have been in this part of the world at an early enough date to have taken the Sacramento Mts. *S. nokomis*.

There are, however, two historical records from the Sacramento Mts. that I personally accept (Toliver *et al.* 2001). One is from Fort Stanton, site of the infamous incarceration of our Apache and Navajo people in the1860s and 1870s. This site is now so disturbed that it is impossible to tell precisely where a *S. nokomis* colony once existed, as all the groundwater has been tapped for human follies, ranging from "taming" wild Indians to taming tuberculosis. The United States established Fort Stanton, Lincoln County, in 1855 (Julyan 1996).

The other record is from Bent in Otero County on the dramatic 3000-foot western escarpment of the Sacramento Mts. Apparently a now-capped underground stream once flowed out of the escarpment here in a scenario reminiscent of the well-known Sierran Round Valley home of the *Speyeria nokomis apacheana* (Skinner) colony in Inyo County, California. The Bent area was slightly more recently settled than Fort Stanton, probably around 1870—it was likely named after the family of Governor Bent, the first American governor of New Mexico, who was assassinated in 1847. Bent is sort of a suburb of Tularosa, the oldest European outpost in the Tularosa Valley. Specifically, Julyan (1996) states, "In 1862 Hispanic settlers arrived at the edge of the marshy land where Tularosa Creek fans out and loses itself among reeds and marsh grass about a mile from the mouth of Tularosa Canyon." It is highly probable that this marshy area was once the aboriginal home of the Sacramento Mts. *Speyeria nokomis*, and, as the water was tapped, the butterfly retreated up Tularosa Canyon, making a last stand at Bent.

Another Lepidopteran also dependent on a copious desert water supply, *Papaipema dribi* (Barnes & Benjamin), was also known from a similar site at High Rolls, about 20 miles south of Bent, but now is presumably also extirpated (Barnes & Benjamin 1926). (J.R. Wiker, caretaker of the Moth Photographers' *Papaipema* section, has informed me that no *P. dribi* have been taken anywhere since the type series (one pair), which is housed at the United States National Museum (Wiker pers. comm.). High Rolls, in Fresnal Canyon, differs from Bent in being about midway down the Sacramento West Escarpment instead of near its base. Both Tularosa and Fresnal Canyon drain into the closed Tularosa Basin, which includes White Sands National Monument and Missile Range.

I, thus, believe the *Speyeria* population in question was observed and extinguished between 1855 and1900,

and most likely between 1885 and 1900.

As alluded to above, Cary had determined that it would have been physically possible for the early New Mexican collector W. J. Howard to have been at the above sites before 1890 without appealing to time travel. I am not stating that the *Speyeria* records are due to Howard, or that there is any evidence Howard ever visited either site—I merely point out that we do not need to add a sixth problem to the above list. We can, at least, say that someone with a butterfly net could have been in the Sacramento Mts. at the time demanded. Moreover, Howard may have spoken to some unknown third party—possibly a soldier stationed at Fort Stanton—and encouraged him to write home about the butterflies or even to catch some. There is some chance Henry Viereck could have been the primary source of the Bent records—he is known to have collected at High Rolls at least as early as 1902 (Cary and Holland 1992).

I do think that *S. nokomis* was gone, at least from Bent, by 1900, because Bent is not that far from Las Cruces, where a world-class naturalist, T. D. A. Cockerell, then resided (Weber 1976). Cockerell described two varieties of *S. nokomis* from northern New Mexico that decade, and it seems unlikely that anything this spectacular would have totally escaped his purview (Cockerell & Cockerell 1900; Cockerell 1909). By 1900, reports of *S. nokomis* were even being published from the Sierra Madre in Chihuahua (Holland 1900).

At this point, I need to explain my personal involvement with this enigma. In July 1965, just before I relocated from Boston to Albuquerque, I spent a weekend with Paul Grey, co-author of the landmark *Speyeria* revision (dos Passos & Grey 1947) reducing the species number of American *Speyeria* from 109 to 13. Both Sacramento Mts. reports for *Speyeria nokomis* that I consider credible reached me orally from Paul Grey; he told me, in person, of the old records from Bent and Fort Stanton. He suggested I try to confirm them. In 1965, I did not know this was going to lead to the ultimate New Mexican butterfly mystery.

In summary, the historical reality connecting us to a Sacramento Mts. *Speyeria nokomis* is less than that connecting us to the Lost Continent of Atlantis. In Plato's writings, there is at least a quasi-contemporary third-person mention of Atlantis (see the dialogues Timalos and Kritias (Plato 355 BC)), so only the first four of the five above problems lie between us and Atlantis. On the other hand, dos Passos & Grey's revision does not even mention the Sacramento *nokomis*. My contribution here is to evaluate the credibility and the judgment of the three scholars in this matter whom I have known personally (and to add my own experiences).

It is also my objective to place my personal intuition and experience in a position that obstructs application of the 100-year rule about oral tradition to the validity of these old records. This note is intended to undermine Objection 3 in this case–I think Paul Grey probably did know someone who saw the genuine article.

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The list of persons touched by this enigma is unparalleled in the annals of the study of our Lepidoptera–it reads like a who's who of the best of us: I acknowledge Fred Rindge, Mike Toliver, John Rawlins, T. D. A. Cockerell, F. M. Brown, Harry Clench, Otto Poling, Paul Opler, Henry Vicreck, Paul Grey, Cyril dos Passos, Paul Ehrlich, Steve Cary, Chuck Bridges, Joanna McCaffrey, W. J. Townsend, Prof. Snow, W. J. Holland, and one anonymous source.

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POSTSCRIPT

After this article had been submitted, I tried a rather unlikely final attack on the Sacramento Mountains mystery. I knew that Paul Grey's *Speyeria* collection at the AMNH had always been separately maintained and not integrated into the rest of the AMNH holdings—largely due to its unique and pivotal role in the history of parsimonious taxonomy. Consequently, I wrote there and explicitly asked the current curator to search the Paul Grey collection for the Holy Grail. In reply, I received the astonishing news that *two specimens had been discovered as a result of my inquiry*. As I had predicted, they were taken in Bent, but considerably more recently than my guess of 1900 for the extinction of the population. Specifically, two males turned up, one bearing the label "New Mexico, Otero Co., vic. Bent, ex Ehrlich Coll." and the other bearing the label "Bent, Otero Co., New Mex. Aug. 12, Ex. Coll. Ehrlich" plus the additional label "Coll. of L. P. Grey". All three labels to me appear to be in Paul Grey's hand. Both are *Speyeria nokomis nokomis*. To

Suzanne Rab Green, AMNH doeent, I owe a debt beyond measure, as it was she who aetually located the specimens.

Not wishing to be overshadowed, this discovery caused the CMNH people to dive into eabinets unexplored sinee the demise of the .400 batter, wherefrom they surfaced with two specimens labeled "Mescalero, N. Mex. VIII 13, 1931, W. Huber" and "Exch. A.N.S.P., C.M.Ace.20359." On one of the labels, the VIII is just VII. I suggest this is probably a misprint, as it is unlikely these treasures would have been taken exactly a month apart. The second label refers to an exchange of vertebrates for invertebrates that took plaee between the Carnegie Museum and the Philadelphia Academy of Seiences around 1940. These are female *Speyeria nokomis nr. nokomis*. W. Huber was head mammalogist at the Philadelphia Aeademy of Seienees in 1931, and regularly spent summers doing fieldwork around Tularosa, New Mexieo. Sinee the reliability of the Ehrlichs, Paul Grey, and W. Huber is absolute, it now appears this greatest of all New Mexico butterfly mysteries is resolved. I have wondered if this Ehrlich collector was the Paul Ehrlich we all know and love, or rather a relative. (The Paul Grey collection was transferred to the AMNH in 1948, when our Paul Ehrlich would have been about 15—rather a young age at which to have eollected the material, donated it to Paul Grey, and have had it redonated to the AMNH). I contaeted Paul Ehrlieh and he reports having no knowledge of the material. There is no year of eolleetion noted on the Ehrlich specimens. Photos of both sexes of this population are attached without comment (Fig. 1) — other than a huge thanks to Ms. Green and John Rawlins.

I am also greatly in debt to one of my tenants, Wenyun Zuo, a visiting bio-ecology graduate student at the University of New Mexico. Ms. Zuo made the initial personal contaet with the Carnegie Museum while visiting Pittsburgh in May of 2007. Wendy's personal charm contributed greatly to initiating this search on a very positive note. But by far the greatest help has come from my life partner, Martha Romero, whose assistance with living, running a business, and becoming an instant expert on Lepidopteran literature and curation has kept me productive far into the ravages of Parkinson's disease.

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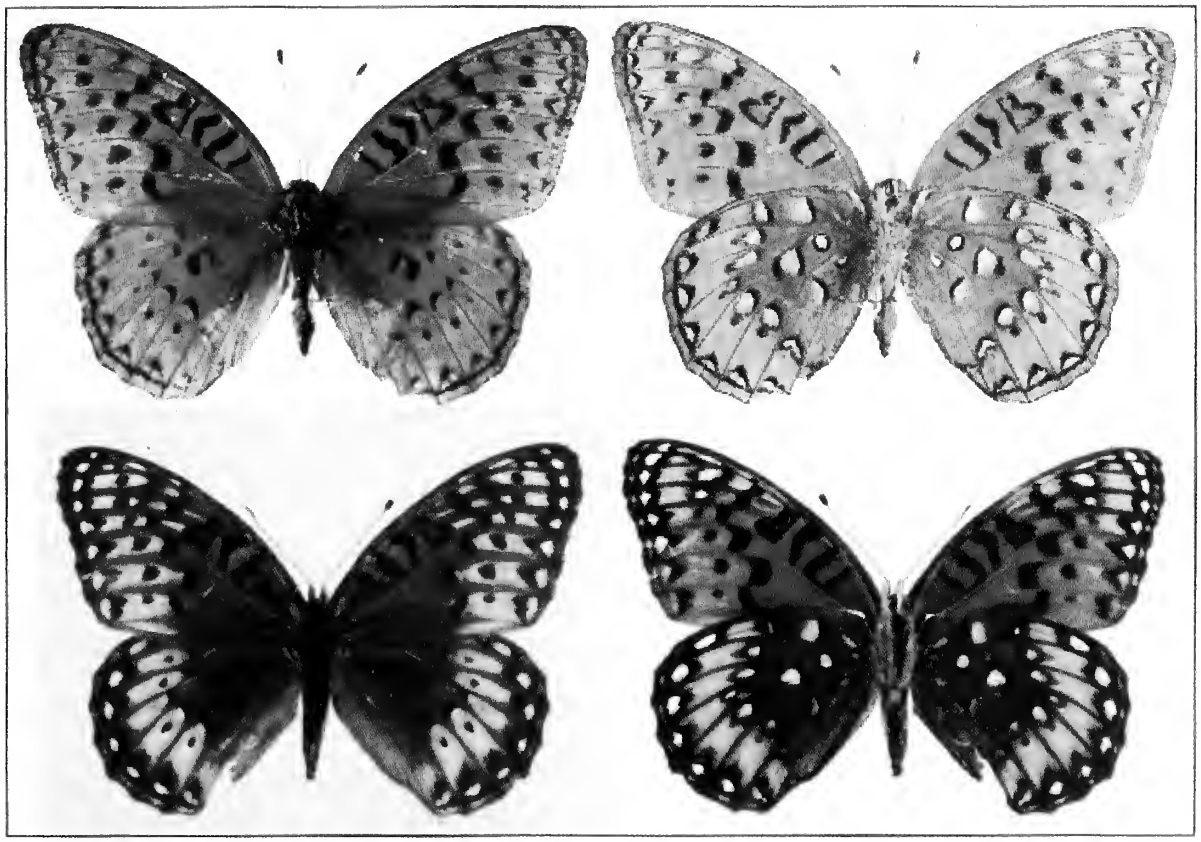

FIG. 1. The Sacramento Mountains population of *Speyeria nokomis nokomis*, now presumably extinct. Top: male, Bent, Otero County, New Mexico, ca 6000', Aug. 12, AMNH collection, ex. Paul Grey Coll, ex. Ehrlieh collection. Year is not specified, but Paul Grey donated his collection to the AMNH in 1948, placing a clear minimum age on the insect. Bottom: female: Mescalero, Tularosa River, Otero Co, New Mexico, ca 7000', July 13, 1931, leg. W. Huber, CMNH collection, ex Philadelphia Academy of Seiences Collection. There is evidence the female may have been taken Aug. 13, 1931.

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AN OBSERVATION OF *CYMAENES TRIPUNCTUS* (HESPERIIDAE) IN NORTH-CENTRAL FLORIDA

Cymaenes tripunctus (Herrich-Schäffer) (Fig. 1) is a widely distributed species occurring in southern Florida, the West Indies and southward to Argentina (Pyle 1981; Cech & Tudor 2005). The precise range of *C. tripunctus* in Florida has been difficult to determine due to its secretive habits, as well as confusion and misidentification, in both the field and museum collections, with the similar *Lerodea eufala* (W. H. Edwards) and *Nastra neamathla* (Skinner & R. C. Williams) (Smith *et al.* 1994; Glassberg *et al.* 2000). Long antennae, approximately one-half the length of the forewing, distinguish *C. tripunctus* from other similar-sized dark-colored hesperiid species (Opler & Krizek 1984; Smith *et al.* 1994; Glassberg *et al.* 2000).

Kimball (1965) only reported a few records of *C. tripunctus* from extreme southern Florida and the Florida Keys. In addition, many of the earliest butterfly lists for Florida regarded observations of *C. tripunctus* in the state as dubious or merely representing strays from Cuba (Skinner & Williams 1924; Barnes & Benjamin 1926; McDunnough 1938). Freeman (1942) reported the first verified sightings of *C. tripunctus* from specimens collected as early as 1937 in Miami. The rarity of early *C. tripunctus* records in Florida suggests the species may have only colonized the state in the relatively recent past. Lenczewski (1980) reported *C. tripunctus* as abundant within Everglades National Park. However, Calhoun (1988) encountered *C. tripunctus* in Lee County for first time on 11 December 1983 and suggested the species might be expanding its range northward in the state. Marc C. Minno (pers. comm.) found *C. tripunctus* to be locally common in Broward County in 1982 to 1984. Subsequently, *C. tripunctus* colonies have been encountered locally in Polk (Lake Kissimmee State Park), Osceola (Kissimmee Prairie Preserve State Park) and Pinellas (Fort Desoto State Park, Boyd Hill Nature Center) counties in association with several larval host plants, indicating further expansion of the species northward (Linda Cooper, Lyn Atherton and Tim Adams, pers. comms.). Robinson *et al.* (2002) and Minno *et al.* (2005) identified numerous grass species used by *C. tripunctus* as larval host plants in Florida including *Digitaria ciliaris* (Retz.) Koel., *Tripsacum dactyloides* (L.) L., *Urochloa mutica* (Forsk.) Nguyen, *Paspalum setaceum* Michx., *Panicum maximum* Jacq., and *Setaria macrosperma* (Scribn. & Merr.) K. Schum. (Poaceae). While available food sources do not appear to be a limiting factor for *C. tripunctus*, the cold winters of northern Florida may prevent the species from colonizing further into the southeastern United States.

On 28 September 2002 we observed and photographed a single *C. tripunctus* at Newnan's Lake in Gainesville, Florida (Alachua County) (Fig. 2). Although *C. tripunctus* is listed for Alachua County on the Butterflies and Moths of North America website (Opler *et al.* 2006) (http://www.butterfliesandmoths.org/species?l=2046) this listing appears to be based on

FIG. 1. *Cymaenes tripunctus* at Newnan's Lake, Gainesville, Florida (Alachua County) on 28 September 2002 (Photo: H. L. Salvato).

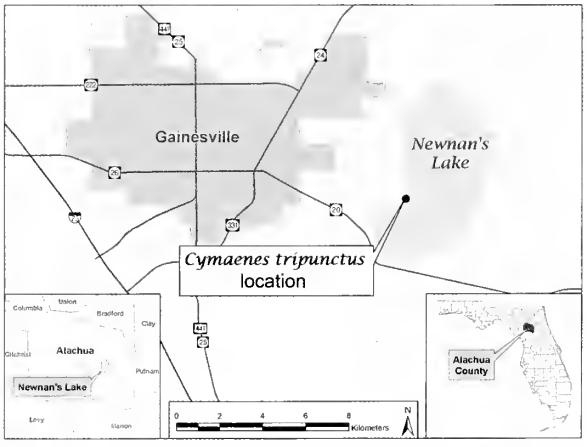

FIG. 2. Location of Newnan's Lake and the *C. tripunctus* observation in Gainesville, Florida (Alachua County).

dubious reports (John V. Calhoun, state records compiler for Florida, pers. comm.). Therefore, to our knowledge this represents the first confirmed observation of *C. tripunctus* in north-central Florida. In the years following our initial observation (2003 to 2007) we returned to Newnan's Lake on several occasions in the late summer and fall to search for *C. tripunctus* but failed to re-encounter the species.

Although we observed no signs of reproduction during our 2002 observation, several host plants for *C. tripunctus*, including *P. maximum*, *P. setaceum*, *Saccharum* spp. and other grass species, occur within the vicinity of Newnan's Lake. Therefore *C. tripunctus* may have established a small temporary population at Newnan's Lake during or prior to the 2002 observation. Further studies are needed to determine if *C. tripunctus* continues to occur at Newnan's Lake. In addition, the status and distribution of *C. tripunctus* should be monitored at the periphery of the species known range to identify any other localized populations.

ACKNOWLEDGEMENTS

We thank Lyn Atherton, Buck and Linda Cooper, Marc C. Minno and Tim Adams for sharing their notes on field observations of *C. tripunctus* and its hostplants at various locations in central and southern Florida. We thank John V. Calhoun for assistance in determining historical locations for *C. tripunctus* at the northern edge of the species range in Florida, as well as for his assistance and enthusiasm in helping us to obtain numerous papers, including many older publications, on the species. We thank J. V. Calhoun, B. Scholtens and M. C. Minno for comments that helped to improve the manuscript. We also thank Barry Wood for preparing Figure 2.

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EUCHROMIINI DE GUYANE FRANÇAISE (LEPIDOPTERA : ARCTIIDAE, ARCTIINAE). By Jean-Aimé Cerda. 172 + IV pages, 1 color map and 3 color plates inserted; 2 CDS of images. ISBN 978-2879780 61-0. Paper back; 29.5 X 21 cm. Published by the author in 2008. Available from him for 60 Euros at: SARL Patawa, B.P. 98, 97357 Matoury, French Guiana (patawa2@wanadoo.fr). The Euchromiini are fascinating wasp mimics that are not often observed in abundance. The group is mostly neotropical, being distributed from northern Argentina to southern USA (CA, TX, FL). Except for a few recently revised genera (e.g. Dietz 1994, Simmons & Weller 2006), many taxa are difficult to identify due to a lack of modern literature, their last global treatment dating back to 1916–1917.

This impressive work treats all 136 species of Euchromiini so far encountered in French Guiana (FG) or mentioned from the country. Many new taxonomic and nomenclatural acts are provided, i.e. 12 new species, 18 new synonymies, 26 new combinations, and 7 names for which the status is revised. The higher classification follows that of Kitching & Rawlins (1998). The genera are classified according to the French version of Draudt (1916–1917) and the species are presented in chronological order by publication date.

The book begins with a detailed abstract. This is followed by an introduction that deals with the earlier comprehensive works on Euchromiini, the tribe's distribution within FG, and a list of the numbers of species found in each of the country's 17 zones. One page is then devoted to the contents of the book, which lists the collections from which specimens were examined and explains the contents of the species treatments. On the next page there are lists of species withdrawn from the faunal list of FG, of new species for the fauna of the country, of species known only from FG, and of the numbers of species that FG shares with other countries or Brazilian states. The taxonomic treatments follow, starting with the genus *Phaeosphecia* Hampson. In the case of this genus and some others there are notes following the mention of the genus and type species names.

For each new species the description is followed by information on types, etymology, notes (sometimes), and black and white figures of the male genitalia. In addition, the upper- and undersides of each new species' habitus are presented in color on three plates inserted between pages 106 and 107. The other species accounts are provided with a synonymic list and the most important citations of their name(s), frequency and method of capture, and a list of collecting localities with flight period in FG by zones indicated on a color map inserted after page 106. In addition, for many species there are historical notes on the concepts of the synonyms, other notes (e.g. on type specimens), a special mention of species new to FG, and a list of localities where non-FG specimens originated. The habitus and male genitalia of all species are represented in color on two separate cd-roms. The female genitalia are not treated at all in this work. The general distribution of all FG species is depicted on 14 maps with the borders of all neotropical countries and states of Brazil delimited.

The book ends with reasons why seven species names were deleted from the country's faunal list, acknowledgements, a bibliography, an index to taxon names, and an index to the species names on the maps.

I was impressed by the quantity of work that went into this book. The illustrations are of good quality, although the printed black and white genitalia figures would have benefited from more contrast. There are usually five figures of male genitalia for each species to show important characters in focus as the depth of field of each image is narrow.

The text is partly translated into English, making the book easier to consult for a wider audience. Specifically, the parts translated are the abstract, introduction, contents, notes under each genus and species, notes on abundance and collecting methods for each species, and the text pertaining to the species deleted from the faunal list of FG. The descriptions and diagnoses of the new species are not translated. In general the quality of the English text is good, but there are spelling mistakes.

In my opinion *Euchromiini de Guyane française* would have benefited from the addition of a checklist, more elaborate and translated diagnoses for new species, the addition of diagnoses for all other species, more information on females, some introductory information on the morphology and monophyly of the group, and a thorough read by an experienced taxonomist. However, the author must be congratulated for his work. It will be indispensable to all interested in euchromiines and it should be added to all museum libraries containing decent collections of these beautiful moths.

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